ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1965-1966

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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ANNUAL REPORT of Program Activities

United States of

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

(July 1, 1965 through June 30, 1966)

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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

ANNUAL REPORT

July 1, 1965 through June 30, 1966

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NICHD Annual Report
July 1, 1965 through June 30, 1966
Office of Director

Introduction

Since the establishment of the National Institute of Child Health and Human Development in January 1963, its evolutionary process has been as fluid as the continuum of the human developmental processes it seeks to define. During this past year, the results of some of the thinking and planning which have gone into the transition of basic organizational and programmatic patterns have become visible. As the intramural and extramural activities have been further defined and developed, so have the organizational structure, program policies and institute activities been modified and expanded.

By supporting, conducting, and stimulating research and research training, the Institute hopes to achieve its continuing objective, which is to relate all of the sciences to the processes by which human beings can be conceived, reared, and developed in a way which enables them to achieve their optimal potential throughout their entire life spans. Such research is also necessarily concerned with the isolation of those factors which may hinder or damage this optimal development. These broad objectives require that each program be approached from many different points of view at each point in time, and at continuing points in time. Thus the research and training programs are planned not only to produce the knowledge we need to understand fully a developmental process, but also to improve the quality as well as the quantity of those investigators whose points of view are now relevant, or may become relevant, to a full understanding of how people develop and function.

The number of investigators and administrators in the United States who understand the implications of both interdisciplinary and multidisciplinary research as it can be applied to specific unknowns in human development is not large. One of the missions of the Institute, therefore, has been not only to identify such investigators, but also to develop such investigators from among those who have achieved competency in specific fields. This has meant, in part, training young and enthusiastic men and women from many disciplines to work together on studies leading to an understanding of, and therefore the eventual improvement of, human development. If the Institute's mission is to be accomplished, it is essential that the time-relatedness of developmental phenomenon be delineated, so that, for example, the relationship between investigations into reproductive success or failure of parents can be meaningfully correlated with the adult performances of their children.

In order to provide the framework in which this kind of program can be most effectively administered, it was found that a reorganization was necessary to provide a maximum of interdigitation of the most interrelated disciplines and programs, and, at the same time, to maintain an identification of some of the more pressing areas of research. To provide the Institute with as

much program planning and policy consultation as was needed in areas which have been difficult to develop and in which the Council believes more emphasis is needed, special nongovernmental expert committees have been formed and specific conferences held. Active and continuous recruiting for the highest quality staff has been successful within the limitations of the salary and research opportunity competition with the academic community. Rapid expansion during this year of the intramural research opportunities through transfer from other institutes of appropriate program activities and through the creation of new facilities has expanded the potential for research not now being emphasized elsewhere, and for the recruitment of staff eager and equipped to carry it on. The interest and activity of the Council has greatly supported the staff in its efforts to develop and staff programs demonstrating this complex approach to research into the intricacies of why children and adults are the way they are, thereby providing the basis for offering to society the alternatives which possibly can make them better.

Organization

As the Institute developed over its first three years, it became increasingly evident that the Institute's objectives could best be carried out by an organization that placed principal emphasis on major scientific research areas. Such an organization was recently approved by the Surgeon General. The new organization places principal emphasis on four scientific program areas: Reproduction, Growth and Development, Aging, and Mental Retardation.

Under the general direction of the Institute's Director, the Scientific Director provides overall direction and guidance to the four scientific programs. Each program area is under the leadership of a Program Director who develops, plans, and conducts both intramural research activities and the scientific aspects of extramural programs, giving unified direction and leadership by scientific program.

The Reproduction Program is concerned with clinical, biological, and behavioral aspects of animal and human reproduction. All of the problems of fertilization, pregnancy, congenital malformations, and perinatal biology will be included in the scope of the program.

Recommendations on the role of the National Institutes of Health in supporting research on fertility, sterility, and population dynamics were unanimously adopted by the National Advisory Child Health and Human Development Council and approved by the Surgeon General of the Public Health Service.

The Growth and Development Program is concerned with all the factors accounting for differences in development at any time up to the achievement of adulthood. Therefore biological, psychological, social, and cultural considerations are included in the program.

The Aging Program is concerned with the biological, behavioral, and social aspects of aging including the study of the progressive changes that take place in cells, tissues, organ systems, individuals, or groups of individuals with the passage of time after adulthood has been achieved.

The Mental Retardation Program deals with all aspects of retardation from the biomedical, behavioral, and social points of view. In cooperation with the Division of Research Facilities and Resources, the program also administers the program of construction and support of centers for research in mental retardation and related aspects of human development.

In addition to defining the four major scientific program areas, the organizational structure of the Institute provides for three Associate Directors. Technical and administrative services are provided by the Associate Director for Program Services and his staff. Under the Associate Director for Program Services are the Extramural and Contract Management Branch, the Facilities and Resources Branch, the Program Statistics Branch, and the Epidemiology and Biometry Branch.

The Associate Director for Planning and his staff are responsible for projecting needs and necessary future activities of the Institute.

Finally, provision is made in the reorganization for an Associate Director for Communications, within whose area are the Public Information Branch, the Interdisciplinary Conference Branch, and the Scientific Information Centers Branch. Under the overall guidance of the Associate Director for Communications, these branches conduct the Institute's programs of scientific, technical and public information activities.

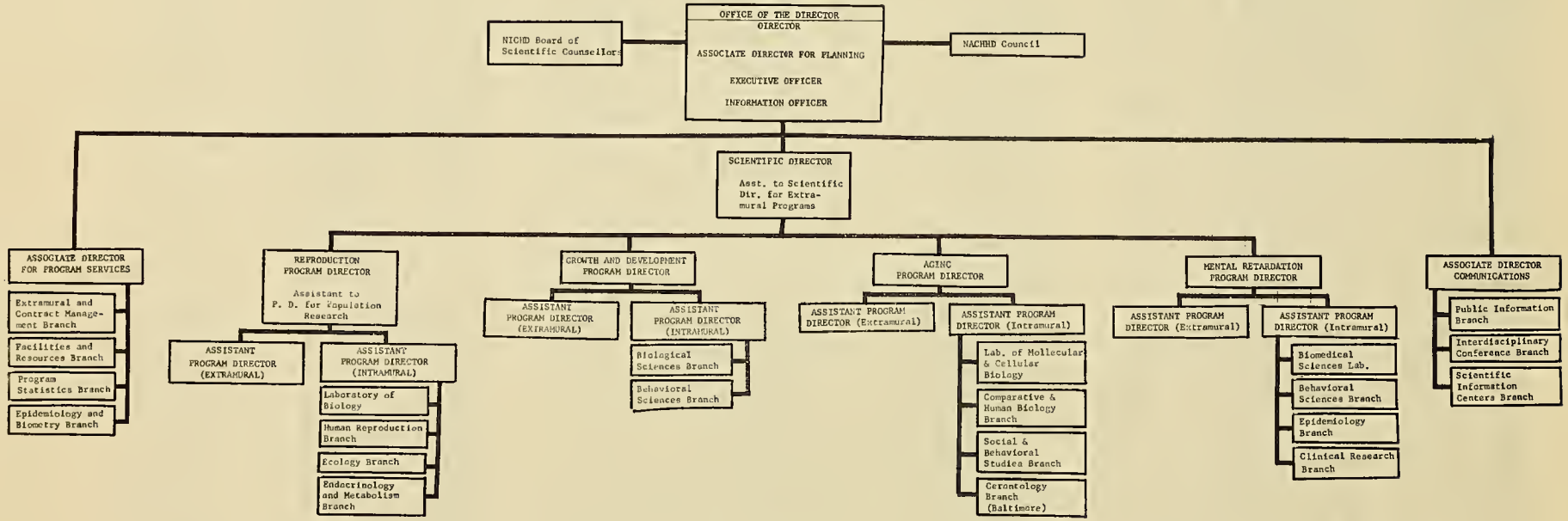
The Institute has established special committees with non-federal scientists to assist it in developing its program and administering its grants programs.

The Special Program Planning Committee was established in 1964 to review scientific areas important to NICHD program responsibilities and make recommendations concerning Institute objectives to the NICHD Director. While it has nothing to do with approval of grants, the committee's work may well stimulate grant applications as well as intramural research. In its first six meetings, four of which were held this fiscal year, the committee dealt with research problems in learning, fertility, differentiation, processes of mammalian pregnancy, aging, and the child in society. The final session was followed by an executive meeting to formulate a final report to the Director which the committee hopes to complete this summer.

Three training review committees for Aging, Reproduction, and Child Development and Mental Retardation review all training grants, career awards, or fellowships pertinent to the interests of the program areas with which each committee is concerned. Besides enabling the research training effort to be focused on program areas, these committees help the Institute plan for future training efforts, taking into account the scarcity of fully qualified research personnel in many fields important to the Institute's programs.

ORGANIZATION CHART

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT



Intramural Research

Initially, and during its first two years of existence, the Institute placed its major emphasis on developing and administering, soundly and imaginatively, its research and training grant programs.

During the year just past, however, a firmly based intramural research program has been achieved. This was brought about by the transfer of several activities from other parts of the National Institutes of Health, and the opening of a new mental retardation clinical facility.

The transfer of the Gerontology Branch of the National Heart Institute to the NICHD represents the largest single action taken to develop the intramural program. The research and training grants in gerontology formerly supported by the other Institutes were transferred to NICHD in 1963. With the Gerontology Branch transfer, all major aging research and training activities at NIH are now consolidated in the NICHD.

Located on the grounds of the Baltimore City Hospitals, the Gerontology Branch is continuing to emphasize investigations of the basic biological mechanisms of aging and the description and interpretation of age changes in the various organ systems of human beings, as well as overall changes in performance and behavior. It is also continuing and expanding its longitudinal study of more than 500 men from all walks of life who come to Baltimore every 18 months for a complete 2-day examination.

Construction has started on a new four-story Laboratory building for aging research on the grounds of the Baltimore City Hospitals. Due to be completed in 1967, this building will provide a badly needed research facility housing approximately 300 professional and ancillary personnel. When completed, the facilities and resources available at Baltimore will be the most comprehensive in the country and will serve as a regional focal point for research in aging.

Two major intramural research units--the Endocrinology and Metabolism Branch and the Laboratory of Biology--were added to the Reproduction Program as a result of a transfer of the major part of the National Cancer Institute's Endocrinology Branch to the Institute.

The NICHD Endocrinology and Metabolism Branch carries out clinical research projects concerned with the genesis, course, and treatment of problems in reproduction and allied disorders. The Branch has a 13-bed research ward in the NIH Clinical Center for the care, treatment, and study of patients.

The Laboratory of Biology conducts basic research related to the control of sex gland secretions, the nature and effects of fetal hormones on the reproductive tract, and the mechanisms regulating reproductive processes in monkeys and other laboratory animals.

With the completion of remodeled facilities at the National Naval Medical Center, the Mental Retardation program began operating, on a cooperative

basis with the U.S. Naval Hospital, an outpatient Diagnostic and Study Unit. This facility provides a program of clinical research and a source of case material for basic research in the biomedical and behavioral aspects of mental retardation. While the Study Unit functions as a direct research activity of the Institute, it also provides, as a byproduct of the research and training conducted, an important service to the dependents of military personnel in the Washington, D. C. area.

Currently, more space adjacent to the Diagnostic and Study Unit is being renovated as a Biomedical and Behavioral Research Laboratory for work in the field of mental retardation.

It is expected that the NICHD Intramural Research activities will continue to grow in the foreseeable future. The Institute has been working closely with the National Institute of Neurological Diseases and Blindness to plan and develop a joint research facility in Puerto Rico, primarily to conduct research in primates. This Institute's activities, established in conjunction with the NINDB's program of research in the development of the central nervous system, will focus quite heavily in the area of basic reproductive biology and behavior relating to reproduction.

In addition, planned remodeling in the Clinical Center at Bethesda will make permanent room available for the Institute's activities in the Reproduction Program. When this space becomes available, one of the first projects of this group will be the establishment of an interfertility clinic to investigate the emotional and psychological as well as the hormonal aspects of infertility. Emphasis upon the problem of infertility rests on the fact that one couple in six fails to have children.

One final note concerning the intramural program: the Institute's Developmental Science Laboratory Building, to be constructed on the National Institutes of Health grounds, is now about to enter the design phase.

Research and Research Training Grants

The most recent data show that the NICHD grant portfolio continues to grow. In March of 1965 active grants of all types numbered 1126; by March 1966 they had increased to 1352, a gain of one-fifth. The dollar amount of grants, meanwhile, rose from 31 million to 41 million, an increase of nearly one-third. (See Tables 1 and 2).

Fellowships and training grants showed the largest increase - each of these programs was more than half again as large in March 1966 as a year earlier. The research grant program grew by more than a fourth during this period. The training programs - training grants, fellowships, and research career program - now make up almost one-fourth of the Institute's grant program.

About eighty percent of the NICHD extramural program is concentrated in two program areas - Reproduction and Growth and Development; the Reproduction Program accounts for approximately half the total Institute grant program, and the Growth and Development Program about a third. (See Table 3).

TABLE 1
NICHD Appropriation History
(in thousands of dollars)

	1964 Appropriation		1965 Appropriation		1966 Appropriation		Pres. Budget 1967 Request	
	Pos.	Amt.	Pos.	Amt.	Pos.	Amt.	Pos.	Amt.
Grants								
Research Grants		$26,093		$30,762		$38,081		$41,817
Fellowships		2,364		2,600		3,707		3,800
Training Grants		4,142		5,544		7,937		8,119
Subtotal, Grants		32,599		38,906		49,725		53,736
Direct Operations								
Direct Research	15	120	97	1,632	192	2,809 1/	204	3,972 2/
Collaborative Studies	44	427	50	937	70	1,936	70	2,071
Training Activities	0	--	3	60	10	90	10	119
Review and Approval	33	431	43	555	56	662	58	751
Program Direction	45	423	54	597	62	698	62	799
Subtotal, Dir. Operations	137	1,401	247	3,781	390	6,195	404	7,712
Total, NICHD	137	34,000	247	42,687	390	55,920 1/	404	61,448 2/

1/ Includes Comparative Transfer of 85 positions and $896,000 from NHI for Gerontology Branch

2/ Includes Transfer in the Estimates of 85 positions and $897,000 from NHI for Gerontology Branch

TABLE 3

NICHD Grants, Active March 1966

By Program Area and by Type of Grant

Program Area	Number of Projects					Current Support				
	Total	Res.	Tr.	Fell.	RCP	Total	Res.	Tr.	Fell.	RCP
Total	1,352[a]	1,035	102[a]	137	78	$40,902,876[a]	$31,624,448	$6,497,447[a]	$1,176,923	$1,604,058
Reproduction	720	568	42	75	35	18,705,778	14,337,097	3,017,761	625,095	725,825
Growth & Develop.	448	331	39	46	32	13,876,214	10,465,550	2,346,973	415,411	648,280
Aging	84	63	9	7	5	4,121,908	3,379,420	577,715	64,286	100,487
Mental Retardation	98	73	10	9	6	4,038,976	3,442,381	394,998	72,131	129,466

Percent Distribution by Program Area

Program Area	Number of Projects					Current Support				
	Total	Res.	Tr.	Fell.	RCP	Total	Res.	Tr.	Fell.	RCP
Total	100.0[a]	100.0	100.0[a]	100.0	100.0	100.0[a]	100.0	100.0[a]	100.0	100.0
Reproduction	53.3	54.9	41.2	54.7	44.9	45.7	45.3	46.4	53.1	45.2
Growth & Develop.	33.1	32.0	38.2	33.6	41.0	33.9	33.1	36.1	35.3	40.4
Aging	6.2	6.1	8.8	5.1	6.4	10.1	10.7	8.9	5.5	6.3
Mental Retardation	7.2	7.1	9.8	6.6	7.7	9.9	10.9	6.1	6.1	8.1

Percent Distribution by Type of Grant

Type of Grant	Number of Projects					Current Support				
	Total	RP	GD	AG	MR	Total	RP	GD	AG	MR
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Research	76.6	78.9	73.9	75.0	74.5	77.3	76.6	75.4	82.0	85.2
Training	7.5	5.8	8.7	10.7	10.2	15.9	16.1	16.9	14.0	9.8
Fellowships	10.1	10.4	10.3	8.3	9.2	2.9	3.3	3.0	1.6	1.8
Research Career Prog	5.8	4.9	7.1	6.0	6.1	3.9	3.9	4.7	2.4	3.2

a/ Includes two Chairman's grants not assigned to a program area.

NICHD-Program Statistics Branch
April 12, 1966

TABLE 2
Growth of NICHD Grant Program, 1965-1966

Type of grant	Number of Grants			Amount of Funds		
	Active March 1965	Active March 1966	Percent increase	Active March 1965	Active March 1966	Percent increase
Total	1,126[a/]	1,352[a/]	20.1	$31,153,370[a/]	$40,902,876[a/]	31.3
Research	883	1,035	17.2	24,867,312	31,624,448	27.2
Training	80[a/]	102[a/]	27.5	4,175,429[a/]	6,497,447[a/]	55.6
Fellowships	91	137	50.5	730,465	1,176,923	61.1
Res. Career Prog.	72	78	8.3	1,380,164	1,604,058	16.2

a/ Includes two Chairman's grants not assigned to a program area.

NICHD
Program Statistics Branch
April 12, 1966

Our Institute supports projects in all states but three (Alaska, Idaho, and Wyoming) and in 18 foreign countries. New York State leads the list as the largest recipient, followed in turn by California, Massachusetts, Pennsylvania, and Maryland.

Five institutions - the University of California, Johns Hopkins, Harvard, Yeshiva, and Western Reserve - are currently receiving more than $1 million each in NICHD support. Among them, they account for almost one-fifth of the Institute's grant funds.

The program areas vary in the average size of their research grants--with Aging having an average more than double that of Reproduction--largely because of the impact of program project grants. The Institute average of $230,000 for program projects is almost ten times the average of $25,000 for regular research projects.

Training grants on the average are funded at $63,000. Here too there is substantial variation among the program areas, with the Reproduction average being almost double that of Mental Retardation. In this case, however, the fluctuations may arise, at least in part, from the small numbers of grants involved.

On the whole, averages for fellowships and research career program awards are fairly consistent among the program areas, with the average size of the career award being about 2 1/2 times that of the fellowship award. Postdoctoral fellowships average $7,000 and specials, $11,000; averages for developmental awards are $20,000 and for career awards, $26,000.

Personnel Changes and Additions

National Advisory Child Health and Human Development Council: The appointments of three of the members expired June 30, 1965: Dr. Claude Villee, Andelot Professor of Biological Chemistry, Harvard Medical School; Dr. Wilma T. Donahue, Chairman and Research Psychologist, Division of Gerontology, Institute for Human Adjustment, University of Michigan; and Mrs. R. Sargent Shriver, Jr., lay member. Replacing them as of July 1, 1965 with appointments expiring June 30, 1969, are: Mr. John W. McConnell, President, University of New Hampshire; Dr. Philip M. Hauser, Professor and Chairman, Department of Sociology, University of Chicago; Dr. Richard Blandau, Professor of Biological Structure, School of Medicine, University of Washington.

Dr. Donald Harting, who was serving as Acting Director of NICHD, has been appointed Director.

Dr. Roy Hertz, former Chief of the Endocrinology Branch of the National Cancer Institute, was named Scientific Director of the NICHD. He has not only been responsible for the development of laboratory and clinical research programs but is also Acting Director of the Reproductive Biology Program.

Mr. John C. McDougall has been appointed Associate Director for Program Services; he had been acting in that capacity.

Dr. Joseph M. Bobbitt, who has been Associate Director for Program Planning and Acting Associate Director for Communications, is retiring from the PHS Commissioned Corps.

Dr. Georgia Perkins Reaser, Special Consultant to the Director, has been appointed Acting Associate Director for Communications.

Dr. Ann Kaufman has been reassigned to the Office of the Director as Special Assistant.

Aging Program

Dr. James Birren, Director, has retired from the PHS Commissioned Corps but will continue in an active consulting relationship to the Acting Program Director, Dr. Leroy Duncan.

Dr. Frances Merchant Carp joined the program as Acting Training Officer and Head of Social Gerontology.

Dr. Walter Spieth is now Chief of the Behavioral Sciences activity.

Growth and Development Program

Dr. Roberta Savitz has been appointed Medical Officer and is assisting in the development of the Scientific Information Center for the program. Dr. Walter Henry Glinsman joined the staff as Specialist in Metabolism. Dr. Michael E. Fishman has been appointed as Medical Officer serving as child psychiatry consultant.

Dr. Leon J. Yarrow was appointed as Psychologist in the Cognitive Development Section.

Mental Retardation

Dr. Joseph C. Robinson was appointed as Chief of the Biochemistry Unit. Dr. Beverly White, as Staff Fellow, is assigned to Dr. Herbert Cooper of the Human Genetics Branch of the NIDR to make cytogenetic investigations. Dr. Theodore Tjossem is now Head of the Mental Retardation Research Centers Program. Dr. Eileen Barker has joined the staff of the Mental Retardation Diagnostic Unit at the National Naval Medical Center. Dr. Paul H. Pearson is now Assistant Program Director for Mental Retardation (Extramural Activities). Susan S. Barr is now a Speech and Hearing Consultant in the Diagnostic and Study Unit. Mrs. Alice Abramson joined the staff as an Educational Specialist in the Diagnostic and Study Unit.

Training Review Committee

Dr. James Kavanagh has transferred to head the Training Review Committee for

Child Development and Mental Retardation.

Dr. Francis Kendrick has been reassigned to the Reproductive Biology Program to be in charge of its training review functions.

Dr. Frances Carp is now Executive Secretary of the Training Review Committee for Aging.

Program Services

Mr. Richard L. Hopkins is now Chief of the Extramural and Contract Management Branch.

Mrs. Geraldine Woodward is now Chief of Grants Administration.

Dr. Sarah H. Knutti is now Chief of Facilities and Resources.

Miss Lillian R. Freedman is now Chief of the Program Statistics Branch.

Mrs. Helen Walter has been appointed Supervisory Statistical Assistant in the Program Statistics Branch.

Dr. Priscilla C. Fourt joined the Program Statistics Branch as Biologist.

Mr. David MacKinnon, PHS Commissioned Officer, has been named liaison Architect, Facilities and Resources.

Communications

Mr. Robert Walters is now with the Information Office as Assistant Public Information Officer.

Mr. Fenton Kennedy is now Acting Chief of the Scientific Information Centers Branch and is also Acting Head of the Aging Information Center.

Mr. Albert Bedell is Acting Assistant Chief of the Scientific Information Centers.

Miss Betty Barton, Chief of the Interdisciplinary Conference Branch is now Executive Secretary to the Advisory Council.

Conferences

The professional staff of the Institute uses scientific consultants individually, or more frequently in small interdisciplinary groups, to review specialized problems and identify neglected areas of research and training which should be promoted by the Institute.

An example is the October 1965 conference on neonatal anoxia for the discussion of oxygen administration to premature newborn infants. High oxygen may relieve respiratory distress and hyaline membrane disease, a major cause

of infant mortality. On the other hand, too much oxygen for the premature may cause retrolental fibroplasia resulting in blindness. The high priority needed was established for a miniature instrument to measure oxygen assimillation by such babies in the first days of life. This will be developed by a medical engineering firm under contract from NICHD. Another outcome--guidelines on the use of oxygen for premature infants--must await accurate measurement and more research.

Similar problem-centered interdisciplinary conferences were held during 1965 to help shape research programs in mental retardation, reproduction, growth and development and aging.

A series of one-day seminars on the biological aspects of aging was held under the joint sponsorship of the Institute and the Salk Institute for Biological Studies in La Jolla, California. Developments concerning outstanding recent biological inquiry in the important areas of aging and (1) transplantation, (2) collagen, and (3) endocrinology were presented and discussed. Grantees of this Institute and fellows specializing in aging research shared in the discussions.

In October 1965 a group of pharmacological and biomedical scientists met in an interdisciplinary conference for the purposes of exploring the concept of developmental pharmacology and stimulating research in this field. The primary emphasis of the conference was on the need for more people in developmental pharmacology, an area of major interest to the work of this Institute. Approximately 33 conference participants, experts in a variety of related disciplines, defined developmental pharmacology and identified work that fits within the definition agreed upon. They also identified individuals who are working in developmental pharmacology or closely related work. The conferees agreed that there is enough support available for training programs and that the immediate needs lie in the areas of motivation and recruitment of personnel.

In December 1965 a small conference brought together pediatricians and behavioral scientists to explore and discuss the research potential in the pediatric outpatient departments of teaching hospitals for training and research. Case discussion covered research material relative to the blind, the emotionally deprived child, and the illegitimate child.

Small interdisciplinary scientific conferences are planned for the future to assess the present state of knowledge and probe problem areas in the birth process, infant health, learning, mental retardation, physical fitness, and physical and behavioral adaptation to aging. A scientific summary of the conference discussions is prepared immediately after the meeting for the benefit of the participants and Institute staff as a guide for developing future research programs and setting priorities for training in the respective fields.

During the fiscal year, NICHD has supported a total of twenty-four conferences. Five of these were extramural awards. For these, the initiative and management were carried by the applicant institution. The balance of the conferen-

ces were sponsored or co-sponsored by the Institute, with the involvement of program staff in the planning, execution, and follow-up of each.

By program area, the intramural conferences, for each of which a report is or will shortly be available, included the following:

Reproduction Program

Recognition and Treatment of Neonatal Anoxia

Neonatal Fuel Metabolism

Developmental Pharmacology

Consultation on Population Research

Growth and Development Program

Conceptual and Methodological Approaches to Research in Outpatient Pediatric Departments

Learning Advisory Group Conference

Perpetual Development in Relation to Theories of Intelligence and Cognition
(co-sponsored with the Institute of Juvenile Research, Chicago, Illinois)

Mental Retardation Program

Mental Retardation Research Centers Program Consultation

Prevention of Mental Retardation through Control of Infectious Diseases
(co-sponsored with the University of Texas)

The Inter-American Workshop on Mental Retardation
(co-sponsored with the Department of Health, Education, and Welfare and the University of Puerto Rico)

Aging Program

No conferences were held this fiscal year.

Two specialized conferences on Vocabulary Improvement and Development were held under the auspices of the Scientific Information Centers Branch; one for vocabulary in Growth and Development and Aging, and the second for Behavioral Sciences vocabulary development.

In the course of the three years of the conference program within this Institute, a card index has been developed with biographical information on the

participants. This file, with more than 2000 names, represents a reservoir of talent covering a range of subjects in human development. It also serves as a resource file of younger investigators to be tapped for potential leadership roles in specific research projects.

The experience of the Institute indicates that interdisciplinary conferences can be used to identify and clarify:

a. emergent areas where there is need to delineate a problem to make it operational for research, e.g., developmental pharmacology;

b. technical problem areas where the field is already delineated, but a specific problem needs to be worked out, e.g., neonatal anoxia;

c. continuing subject areas relating to problems which need to be studied again and again, e.g., learning.

Participation in Non-NICHD Sponsored Conferences

There were significant conferences in which NICHD staff participated. At the annual meeting of the Association of Medical School Pediatric Department Chairmen, the topic was, "Manpower". NICHD actively participated in the planning of the first Inter-American Workshop on Mental Retardation, co-sponsored by the Department of Health, Education, and Welfare and the University of Puerto Rico, to delineate mutual areas of interest and need, potential areas for research, and assessment of the need for future international cooperation in program development.

A conference on The Effects of Peacetime Radioactive Fallout on Children and Adolescents, co-sponsored by the USPHS Division of Radiologic Health and the American Academy of Pediatrics defined topics in need of more refined or new research.

Publications

During this year, the NICHD has prepared for publication by the Government Printing Office, four documents which are the result of previous conferences:

INITIATION OF LABOR (Distributed May, 1966)

NEONATAL RESPIRATORY ADAPTATION (To be available Fall 1966)

SUDDEN DEATH IN INFANTS " " " " "

PATTERNS OF HOUSING IN MIDDLE-AGED AND OLDER PEOPLE (To be available Fall 1966).

Scientific Information Centers

During 1966 and 1967 the Institute will establish three Scientific Information Centers for Aging, Growth and Development, and Reproduction. The objectives of these centers is fourfold: (1) to collect and store all information in their respective fields of interest; (2) to be referral centers for information in each field; (3) to analyze and evaluate information so that evaluative reviews can be published periodically; and (4) to keep the researchers aware of the current work beind done in their respective fields of study by means of states-of-the arts' lists.

To date, basic vocabularies have been compiled in the three areas of behaviorals, social, and biological sciences for the aging and growth and development centers. Conferences with specialists in each of these fields have been arranged to improve and polish the vocabularies. Sources of materials have been collected and an information and retrieval system is being designed for programming NIH computer which was installed in 1966.

By June 1966 a full staff for these centers will be hired so that all information in the fields of Growth and Development and Aging can then be indexed, abstracted, stored, retrieved and disseminated. Dissemination will be in the form of abstract journals, special bibliographies, continuing bibliographies and current awareness lists. By the end of 1967, the centers will have enough information stored and staff available so that evaluative reviews can be developed in areas relating to Aging and Growth and Development.

The Scientific Information Center for Reproduction will be in operation in the latter part of 1966 and follow the pattern of the other two centers.

Future Program Plans

A special subcommittee on the Behavioral and Social Sciences of the National Advisory Council on Child Health and Human Development is in the process of preparing recommendations to the Director on ways of expanding and improving the roles of these sciences in the research and training activities of all the NICHD programs, both intramurally and extramurally. Their recommendations were submitted to the Council at its June 1966 meeting.

Reproduction - In 1967 increased support of studies aimed at further understanding of the accelerated rate of population growth, and stimulation of the development of methods for safe and effective controls which will be acceptable to all segments of society is planned. This will include studies of the long-term effects of oral contraceptives and intrauterine devices on maternal health, and the health of children born to women who have used these family planning techniques over long periods of time.

Other areas of emphasis in the coming years are the problems of fetal, perinatal and infant mortality and morbidity. The Institute is seeking new knowledge which will make it possible to reduce the incidence of fetal and infant death and morbidity in all segments of our population. The Institute is cooperating with other government agencies to insure that the advantages

of present knowledge are equally available to all segments of our population, irrespective of socio-economic status.

Increasing effort will be devoted to developing practical ways of reducing fetal and infant deaths and morbidity. Special emphasis will be placed on the relationship between socio-economic status, inadequate nutrition, and perinatal loss. For example information gained in investigations of unfavorable conditions in pregnancy that lead to premature birth will be used to find ways of prevention or correction.

Growth and Development - In 1967 it is planned to expand studies in the processes of learning with consideration for the psychological, neurophysiological, neurochemical, and genetic studies and the effect of social contexts on growth processes. Attention will be given to the development of research on the mother-infant relationship, the effects of deprived environment at different developmental levels and upon the socialization process.

The many complex relationships between nutrition and both physical and mental development are poorly understood. In this area the Institute plans to expand its activities designed to provide more precise knowledge of the role of nutrition in the development of the individual. These activities will encompass not only the usual dietary and clinical aspects of nutrition, but will also include studies of the complex molecular interrelationships of nutrients that support physiological function and control the limits of growth and performance of the whole individual.

Aging - In 1967 the Aging program plans to support research that will lead to an increased understanding of the changes that occur in the physiological functions of man with increasing age. A great deal of information already has been obtained in this field. It is known that the major body systems become increasingly less efficient as the body ages due to a decrease of the efficiency of organs within the systems. Knowledge must be expanded along these lines to increase the quality of medical care of patients. For detailed information of this type, longitudinal and cross-sectional studies will be carried out in which a great many functional systems would be tested and in which appropriate correlations are made. The development of computer technology is making complex studies of this type increasingly practical.

Mental Retardation - The attention focused on the prevention of mental retardation in 1966 by alleviating cultural and environmental deprivation will be intensified in 1967. It is estimated that over five and one-half million children and adults are mentally retarded. Of these, about eighty-five percent fall within the mildly retarded category. Many of these mildly retarded individuals are functioning in the subnormal range because of cultural and environmental deprivation. Since such deprivation contributes to the self-perpetuating nature of the disadvantaged groups in our society, it is extremely important that ways be found to combat it. Research projects will be initiated to determine the environmental and cultural elements contributing to retardation and to discover effective methods for modifying these causes of retardation through improved educational techniques.

Because much of what is identified as mental retardation is thought to be caused by cultural deprivation, the Institute plans to expand its programs concerned with the delineation of the relationship between the two.

NICHD ANNUAL REPORT
July 1, 1965 through June 30, 1966
Scientific Director's Summary

In Fiscal Year 1966, the NICHD was reorganized into three major operating arms: The Scientific Programs under a Scientific Director, Communications Activities under an Associate Director, and Program Services under an Associate Director. The central concept of the reorganization was to place the principal scientific focus on four substantive program areas rather than on administrative mechanisms or scientific disciplines. These program areas, Reproduction, Growth and Development, Aging, and Mental Retardation, are under the general direction of a Scientific Director.

This organization enables the Institute to accomplish its mission more effectively by focusing on the goals of research rather than the means. The substantive Programs are responsible in the respective areas for both the conduct and support of research and training. In furtherance of this concept, broad direction and planning of intramural and extramural efforts are appropriately interrelated by an echelon of senior staff.

The Program Directors who are in charge of each Program area have responsibility for the over-all conduct of the Institute's activities in an entire scientific field and are able to concentrate on scientific program objectives. Also they have available to them a whole range of technical and administrative mechanisms to help them accomplish these objectives. The Program Directors have been challenged by this type of organization which permits them to take part in the development of intramural, extramural and field activities of the NICHD.

An important factor which has a bearing on the organization of NICHD is the broad scope of its program interests. The breadth and diversity of research responsibility impose unique demands on the Institute.

Because of the many approaches to the study of human development, studies will be carried out in a number of disciplines and from varied orientations. It appears that these objectives can best be achieved under this new organization. The Program Director concept allows a single official, with broad responsibility, to work toward goals in an identified area of concern. He is able to integrate the efforts of his staff in such a way as to gain optimum use of the resources available to him.

Reproduction Program

The Laboratory of Biology and the Endocrinology and Metabolism Branch were created in December 1965 as a result of a transfer of a number of former members of the Endocrinology Branch, NCI, to the newly formed Reproduction Program of NICHD. The transition was not difficult because of the avid interest in reproductive biology developed earlier under the leadership of Dr. Roy Hertz, who became NICHD's first Scientific Director.

The research activities of the Laboratory of Biology concentrate on problems relating to conception, gestation, interrelationships of the anterior pituitary-adrenal-reproductive systems, and the role of biotin and related factors in the physiology of reproduction. Primate studies by this group have been directed toward systematic, quantitative studies of hormone production and excretion through pregnancy and the menstrual cycle in the rhesus monkey. They are also conducting related studies of ovarian function in the pregnant animal.

The staff of the clinical Endocrinology and Metabolism Branch have united as an effective team for the practice of clinical medicine and the conduct of clinical and laboratory research. Efforts begun by this group while affiliated with the Endocrinology Branch of the NCI have been continued, largely without interruption. Areas of investigative interest include the biological and radioimmunological assays of protein and polypeptide hormones, cytogenetic aspects of endocrine disorders, hormonal induction of ovulation, and follow-up of patients successfully treated for gestational trophoblastic neoplasms.

In collaboration with the NINDB, construction of facilities for investigations on primates has been planned. Laboratories and a primate facility will be constructed at El Centro Medico and Sebana Seca in Puerto Rico. NICHD's activities, established in conjunction with the NINDB's program of research in the development of the central nervous system, will focus on the areas of basic reproductive biology and reproductive behavior.

The Program reorganization within the Institute resulted in the creation of the Reproduction Program by the combination of the former Reproductive Biology, Perinatal Biology, Congenital Malformations, and Developmental Pharmacology Programs. In terms of extramural research and research training, the new Program constitutes about one-half of the NICHD grants. During the year, there was a marked increase in interest in research on population problems. The number of such applications is increasing, but the total investment should be larger. With the advice and encouragement of the Council, the Reproduction Program continues its active programming of foreign research and training grants.

Growth and Development Program

The reorganization of the Institute created a Biological Sciences Branch and a Behavioral Sciences Branch in the Growth and Development Program. In the Nutrition Section of the Biological Sciences Branch, studies being conducted include investigations on the regulation of hepatic protein metabolism and the regulation of carbohydrate metabolism in the isolated rat liver.

In the Personality Development Section of the Behavioral Sciences Branch, research has recently been initiated in certain Washington, D. C., Head Start populations. This project is designed to analyze the components of learning environments of preschool children; to delineate the characteristics of children from deprived environments; to study relationships between specific environmental variables and changes in intellectual and personality

characteristics; and to study the interrelationships between cognitive and personality functions of preschool children.

The lack of laboratory facilities has hindered the development of direct research in the Growth and Development Program. During the next year, a search will be made for available space on or off the Reservation to house activities germane to the mission of this Program.

The Program's interest in the sudden death syndrome has continued. The proceedings of the Conference on the subject, held in 1964, have been published and are being distributed. The MEDLARS printout on the sudden death syndrome continues to go to interested investigators. A review article prepared on contract is before the Editorial Board of a national journal.

The Extramural Program now supports 448 grants amounting to $13,876,214. Approximately 40 percent of the research grants in this Program are concerned with behavioral science research. Extramural programming is ongoing in the broad aspects of molecular biology, developmental immunology, developmental physiology, physical growth, nutrition, physiological correlates of behavior, cognition and learning, social development, personality development and psycholinguistics.

Aging Program

During the Fiscal Year 1966, the Gerontology Branch in Baltimore, Maryland, was transferred from the National Heart Institute to the NICHD. This activity is currently being carried out in laboratories and wards in the Baltimore City Hospitals. A building to house this Branch is currently being constructed and should be ready for occupancy about October 1967.

The goals of the Gerontology Branch are to describe in quantitative terms the changes that take place with aging and to investigate the basic biological mechanisms of these changes with a view to reducing the impairments and disabilities of older people.

Highlights of findings from the Gerontology Branch which have extended knowledge of aging include the following: 1) Older subjects show an impaired response with respect to blood sugar levels to the intravenous administration of tolbutamide. 2) The impairment of glucose tolerance in the aged cannot be ascribed to an increase in free fatty acids in the plasma. 3) Total caloric, vitamin, and mineral intakes diminish with age even in subjects whose diets are not limited by economic factors. 4) A mathematical method has been devised which may make it possible to assess individual differences in non-uniformity of lung ventilation. 5) A double isotope derivative assay for angiotonin has been developed which permits the quantitative estimation of nanogram amounts of the hormone. 6) In rats the ability to make fine discriminations in taste diminishes significantly with age. 7) Age changes in collagen may be due to a reorganization of existing intra-molecular crosslinks rather than the addition of new crosslinks.

The Extramural Program supports research and training for research that bears on the aging process and the developmental and retrogressive changes that occur during the adult years. As of March 31, 1966, there were 63 research grants, nine training grants, seven fellowships, four research career developmental awards, and one research career award being supported by the Aging Program. The total expenditure by the Aging Program was in excess of $6 million. The Program supports a wide range of disciplines that are concerned with various aspects of aging. These disciplines can be divided into four major areas of interest: molecular and cellular biology, comparative and human biology, behavioral sciences, and social gerontology.

The Aging Program has collaborated with the Scientific Information Centers Branch in developing a system that will permit rapid and comprehensive storage and retrieval of information bearing upon aging. This will be achieved by the use of both abstract journals and completely computerized techniques. A wide range of subjects, from cellular biology to social gerontology, will be covered. It is believed that the ability to retrieve information rapidly and comprehensively would be a major aid to scientists in the field of aging.

Mental Retardation Program

Three Branches and one Laboratory have been organized to carry out direct research activities in mental retardation. These include a Clinical Research Branch, a Laboratory of Biomedical Sciences, a Behavioral Sciences Branch, and an Epidemiology Branch. The Clinical Research Branch and the Laboratory of Biomedical Sciences are operational, but the Behavioral Sciences Branch will not be functional until Fiscal Year 1967, and the Epidemiology Branch in Fiscal Year 1968.

The Children's Diagnostic and Study Unit is a Section in the Clinical Research Branch. This outpatient clinic is located at the National Naval Medical Center. The first patient was seen in December 1965, and by the end of the fiscal year, approximately 45 to 50 children will have been evaluated. The purpose of the Children's Diagnostic and Study Unit is to provide a program of clinical research and a source of case material for basic research in the biomedical and behavioral aspects of mental retardation.

Studies are just being started and include investigations on improving attending behavior in the preschool retardate, behavioral assessment of neonates at risk for mental handicaps, and informational discrepancies by parents about their mentally retarded children.

The mission of the Laboratory of Biomedical Sciences is to contribute to the Program's multidisciplinary investigation of mental retardation. Initially, the Laboratory will emphasize biochemistry, cytogenetics, and neurophysiology.

During the past fiscal year, some of the biochemical research has been located in Building 4, NIAMD. The Cytogenetics Unit is located in the

REEL Building at the National Naval Medical Center. Plans for additional laboratory facilities are in the design phase. These additional laboratories will be located adjacent to the Children's Diagnostic and Study Unit at the National Naval Medical Center, and will be ready for occupancy in mid-Fiscal Year 1967.

Projects conducted by personnel in the Laboratory of Biomedical Sciences include pregnancy-associated plasma proteins, electrophoretic variants of leucocyte alkaline phosphatase, studies on hypercalcemia, effects of nutrition on myelinization, effects of amino acids on myelinization, and evaluation of methods of therapy in homocystinuria.

The Mental Retardation Program has primary responsibility for the support of research in the biological, behavioral and social aspects of mental retardation within the context of child health and human development. As of January 1966, the NICHD was supporting 67 research grants, totaling approximately $3.2 million for research directly related to mental retardation. In mental retardation research training activities, the Institute was supporting training grants, fellowships, and research career development awards, amounting to approximately $653,000 at that time. During the past year, research activities continued to focus on three principal areas relating to mental retardation, inborn errors of metabolism, cytogenetics, and behavioral research.

During Fiscal Year 1966, three new Mental Retardation Center construction grants were approved and awarded, bringing to eight the total number of Centers awarded since the initiation of the Program in Fiscal Year 1964. The three construction grants awarded in Fiscal Year 1966 were made to the University of California at Los Angeles, Los Angeles, California; Children's Hospital, Boston, Massachusetts; and the University of Kansas, Lawrence, Kansas.

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July 1, 1965 through June 30, 1966
Reproduction Program

Extramural Programs

During the year covered by this report, program reorganization within the Institute resulted in the creation of the Reproduction Program by the combination of the former Reproductive Biology, Perinatal Biology, Congenital Malformations and Developmental Pharmacology Programs. In terms of extramural research and research training, the new program constitutes about half of the NICHD grants. As of March 1966 the program was supporting 720 grants for a total of $18.7 million. These include 568 research projects ($14.3 million), 42 training grants ($3.0 million), 75 fellowships ($.62 million), and 35 research career and development awards ($.72 million), as indicated in the table.

	Projects		Current Support	
	Number	Percent of NICHD Total	Million	Percent of NICHD Total
Research Projects	568	54.9%	$14.3	45.3%
Training Grants	42	41.2%	3.0	46.4%
Fellowships	75	54.7%	.62	53.1%
Research Career and Development Awards	35	44.9%	.72	45.2%
Total	720	53.3%	$18.7	45.7%

During fiscal year 1966, there was a marked increase in interest in research on population problems. Staff members take advantage of every opportunity to let the scientific community know that the NICHD is interested in supporting good research and research training in this general area. The number of such applications is increasing but the total investment should be a great deal larger. Active programming is continuing and we hope that the results will be reflected in next year's annual report.

With the advice and encouragement of the Council, the Reproduction Program continued its active programming of foreign research and training grants. Two members of the professional staff visited a number of laboratories in

Europe and as a result of these visits two training grant applications and a number of research grant applications have been received. The main emphasis of the Reproduction Extramural Program will continue to be domestic research, of course, but where unique opportunities exist abroad for the support of high quality research and research training in reproduction, we believe that the mission of the Institute will be substantially advanced by such investments.

Intramural Programs

The principal population research carried out by the Reproduction Program of NICHD is conducted by Dr. Roy Hertz and his associates who for a number of years have carried out important research on the endocrinological aspects of reproduction.

1. Dr. Henry Vaillant, an NICHD Research Associate, was assigned through the Population Council to a research project designed to measure the efficacy and safety of the intrauterine device with particular emphasis to its possible relationship to cervical neoplasia. This project is being conducted in Barbados, an area with a high population density and a government favorably disposed to family planning.

2. Our interest in population research has become manifest through the use of contracts. The largest current contract supports the Third Growth of American Family Study. The principal investigator, Dr. Charles Westoff of Princeton, is analyzing the data from the survey conducted last fall and will give a report of his findings in the near future.

3. Another contract (reimbursable agreement) is held by Dr. Harold Hawk of the Department of Agriculture Research Station in Beltsville. Dr. Hawk is carrying out an intensive study of the effect of intrauterine contraceptive devices on the reproductive function of various domestic animals. His findings are significant and extremely interesting. The action of these devices appears to be markedly different in different species. The data from this study not only has direct relevance to fertility control but also indicates that intrauterine foreign bodies may prove to be a useful biological tool.

4. There are several smaller contracts also underway. Of particular interest is one (reimbursable agreement) with the District of Columbia Department of Public Health to determine if it is possible to carry out in the District a long-term study of the safety and efficacy of various contraceptive methods. We hope that the District will prove to be one of several locations where studies of this type may be carried out.

5. The staff of the clinical Endocrinology and Metabolism Branch, consisting of two senior physicians, four clinical associates, two biochemists, four technicians and one secretary have united as an effective team for the practice of clinical medicine and the conduct of clinical and laboratory research.

Efforts begun by this group while affiliated with the Endocrinology Branch of NCI have been continued, largely without interruption.

Areas of investigative interest include the following:

a) Biological and radioimmunological assays of protein and polypeptide hormones

b) Isolation and purification of protein and polypeptide hormones

c) Cytogenetic aspects of endocrine disorders

d) Hormonal induction of ovulation

e) Follow-up of patients successfully treated for gestational trophoblastic neoplasms

f) Application of computer technology to problems in clinical and laboratory investigation

The inter-relations of our individual and joint efforts will be apparent from the discussion of results obtained as the several interest areas are considered in detail. This integration is the spontaneous derivative of a community of interests rather than the result of administratively-imposed, goal-oriented or directed research.

6. Thyroid-stimulating Hormone (TSH)

Estimations of secretion rates of TSH in euthyroid and hypothyroid human subjects have been completed. Reasonably reliable estimates of degradation time and secretion rates of TSH in man have been made for the first time. Results show that in hypothyroidism, secretion rates are increased and degradation time prolonged, accounting for the high levels of TSH observed in the plasma of patients with primary myxedema.

Similar studies have been performed in cattle using a radioimmunoassay for bovine TSH.

7. Luteinizing Hormone (LH)

A radioimmunoassay for luteinizing hormone in man (HLH) has been developed. The method is capable of detecting and quantifying this hormone in volumes of 0.3 ml. of plasma or less and is about 500 times as sensitive as the most sensitive biologic assays.

For the first time levels of luteinizing hormone have been followed in plasma of normal women daily throughout the menstrual cycle. A single sharp peak of activity has been found, occurring about mid-cycle, lasting for 24 hours, and associated with presumptive evidence of ovulation (elevation of basal body temperature and increased urinary pregnanediol excretion). The height of these peaks equals or exceeds levels regularly

found in post-menopausal women. In women with ovaries and irregular menses, minor day-to-day variations have been observed for periods of up to 50 days of amenorrhea. Levels in normal males show minor daily variations and no significant variation has been shown diurnally or in relationship to bed rest or ambulation. In contrast, levels are high in castrate males.

Clomiphene, an agent used in attempts to induce ovulation in anovulatory or oligo-ovulatory women, has been shown to cause an elevation of plasma luteinizing hormone in both men and women. Studies are currently under way to determine the effects of the oral contraceptive agents on plasma luteinizing hormone activity.

Parallel studies, including immunoassays of blood and biologic and immunologic assays in urine, are being carried out in a population of prepubertal children ranging in age from 6 to 9 years. These investigations, in addition to providing information of clinical usefulness in evaluating urinary gonadotropin excretion, should provide some information about the hormonal changes incident to puberty.

The assay methods offer promise in the clinical evaluation of disorders of pituitary and gonadal function.

8. Melanocyte-stimulating Hormone (MSH)

Considerable progress has been made in the development of a radioimmunassay for alpha melanocyte-stimulating hormone. The assay can be carried out with excellent sensitivity and precision in the absence of serum. So far, it has not been possible to demonstrate any alpha MSH in either blood or pituitary tissue of humans but it appears to be present in pituitary tissue of albino rats. The method is not sufficiently sensitive to permit detection of this hormone in the blood of normal rats.

The half-time of disappearance of alpha MSH injected into hypophysectomized rats has been studied by three methods:

a) Following disappearance by biologic assay using hypophysectomized frogs

b) Following disappearance by radioimmunoassay

c) Following disappearance of tracer amounts of radioactively labeled alpha MSH

Data obtained thus far show a half-life of 1.6 minutes by bioassay and of 2.1 minutes by radioimmunoassay. A much longer half-life of 7.8 minutes has been observed in following the disappearance of the radioactively labeled hormone. Studies of the basis for this disparity indicate that the process of labeling alpha MSH results in alterations in the molecule so that biologically it is handled differently than unlabeled hormone. This important observation has considerable pertinence to studies using tracer amounts of

isotopically labeled hormones in determination of plasma half-life, secretion rates, etc.

9. Growth Hormone

A clinical test has been devised for the evaluation of pituitary growth hormone secretory capacity by measuring plasma levels of this hormone before and after the administration of pyrogens. The test has the advantage that pituitary ACTH reserve can also be tested simultaneously by measurement of plasma cortisol levels. This test has been utilized in evaluating patients suspected of having pituitary dysfunction in order to ascertain whether the dysfunction relates to one or more pituitary hormones.

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The research activities of the Laboratory of Biology are being concentrated on problems relating to conception, gestation, interrelationships of the anterior pituitary-adrenal-reproductive systems and the role of biotin and related factors in the physiology of reproduction.

10. Primate studies by this group have been directed toward systematic, quantitative studies of hormone production and excretion throughout pregnancy and the menstrual cycle in the rhesus monkey (Macaca mulatta) as well as related studies of ovarian function in the pregnant animal. In parallel studies on monkeys and rats, we have observed that chemical stimuli for decidua formation in the rodent appear to have no effect in the rhesus monkey. In earlier experiments by this group, it was shown that ovarian hormones are not necessary for normal gestation after the third week of pregnancy, a finding in keeping with observations in man. These marked differences in the reproductive physiology of rodents make it clear that more intensive primate research is necessary.

11. Steroid hormone metabolism in pregnant monkeys is being actively investigated. As a result of improved analytical procedures, especially isotopic tracer methods, thin-layer and gas chromatography, we are now beginning to obtain quantitatively reliable information on steroid hormone excretion. Estrone, estradiol 17-B, and estriol have been identified in monkey pregnancy urine and quantitative methods are now being applied to determine alterations in estrogen excretion during normal pregnancy. It is expected that this data will prove useful in understanding normal and pathological changes during pregnancy.

12. Studies on the nature and effects of fetal hormones are continuing. We have found that the fetal rabbit testis has the capacity to synthesize hormones prior to the time of differentiation of the embryonal reproductive tract. Current efforts are directed at identification of hormones in the gonads of the fetal pig. Knowledge of the actions of fetal hormones should lead to a better understanding of the mechanisms regulating fetal tissue differentiation in both normal and abnormal development.

13. Our investigations on the effects of anterior pituitary hormones on sex steroid production mediated by the adrenal cortex have demonstrated that ACTH in high doses stimulates estrogen production in the ovariectomized monkey. Earlier findings on the adrenal-mediated androgenic action of exogenous ACTH and prolactin in the castrated male rat have been extended by means of hormone-producing pituitary tumors which reproduce the androgenic effects reported earlier. Thus we can stimulate certain aspects of steroid function found in pathological states.

14. The study of biotin distribution in maternal and fetal tissues has demonstrated a marked elevation of biotin in fetal muscle and brain in contrast to the corresponding maternal tissues. Study of cofactor metabolism opens new areas of research in reproductive physiology at the molecular level.

It is to be anticipated that in the coming year we shall continue current research in addition to initiating studies relating to implantation and placenta formation in monkeys and other animals - with a better understanding of the basic processes of reproduction as our goal.

NICHD Annual Report
July 1, 1965 through June 30, 1966
Laboratory of Biology

SUMMARY

The Laboratory of Biology came into being on December 13, 1965 as the result of a mass transfer of 13 former members of the Endocrinology Branch, NCI to the newly formed intramural Reproduction Program of NICHD

The transition was not as difficult as might be imagined because of the avid interest in reproductive biology developed earlier under the challenging leadership of Dr. Roy Hertz.

The research activities of the Laboratory of Biology are being concentrated on problems relating to conception, gestation, interrelationships of the anterior pituitary-adrenal-reproductive systems and the role of biotin and related factors in the physiology of reproduction.

Primate studies by this group have been directed toward systematic, quantitative studies of hormone production and excretion throughout pregnancy and the menstrual cycle in the rhesus monkey (Macaca mulatta) as well as related studies of ovarian function in the pregnant animal. In parallel studies on monkeys and rats, we have observed that chemical stimuli for decidua formation in the rodent appear to have no effect in the rhesus monkey. In earlier experiments by this group, it was shown that ovarian hormones are not necessary for normal gestation after the third week of pregnancy, a finding in keeping with observations in man. These marked differences in the reproductive physiology of rodents make it clear that more intensive primate research is necessary.

Steroid hormone metabolism in pregnant monkeys is being actively investigated. As a result of improved analytical procedures, especially isotopic tracer methods, thin-layer and gas chromatography, we are now beginning to obtain quantitatively reliable information on steroid hormone excretion. Estrone, estradiol 17-β, and estriol have been identified in monkey pregnancy urine and quantitative methods are now being applied to determine alterations in estrogen excretion during normal pregnancy. It is expected that this data will prove useful in understanding normal and pathological changes during pregnancy.

Studies on the nature and effects of fetal hormones are continuing. We have found that the fetal rabbit testis has the capacity to synthesize hormones prior to the time of differentiation of the embryonal reproductive tract. Current efforts are directed at identification of hormones in the gonads of the fetal pig. Knowledge of the actions of fetal hormones should lead to a better understanding of the mechanisms regulating fetal tissue differentiation in both normal and abnormal development.

Our investigations on the effects of anterior pituitary hormones on sex steroid production mediated by the adrenal cortex have demonstrated that ACTH

in high doses stimulates estrogen production in the ovariectomized monkey. Earlier findings on the adrenal-mediated androgenic action of exogenous ACTH and prolactin in the castrated male rat have been extended by means of hormone-producing pituitary tumors which reproduce the androgenic effects reported earlier. Thus we can simulate certain aspects of steroid function found in pathological states.

The study of biotin distribution in maternal and fetal tissues has demonstrated a marked elevation of biotin in fetal muscle and brain in contrast to the corresponding maternal tissues. Study of cofactor metabolism opens new areas of research in reproductive physiology at the molecular level.

It is to be anticipated that in the coming year we shall continue current research in addition to initiating studies relating to implantation and placenta formation in monkeys and other animals - with a better understanding of the basic processes of reproduction as our goal.

It is a pleasure to express our appreciation for the moral and material support provided by the NICHD staff.

Serial No. HD-RP1
1. Laboratory of Biology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Physiological and Pharmacological Studies of Reproductive Processes in Primates

Previous Serial No.: None

Principal Investigator: Dr. William W. Tullner

Other Investigators: Dr. Roy Hertz, Dr. Amos Palmer, Mr. Bill Hopper

Cooperating Units: Laboratory of Pathology, DBS

Man Years:
Total: 5
Professional: 1½
Other: 3½

Project Description:

Objectives:

1. To gain basic information concerning hormonal changes occurring during gestation in primates.
2. To gain basic information on interrelationships of the anterior pituitary-adrenal-reproductive system.
3. To evaluate the effects of mechanical and chemical factors in the process of deciduoma formation.
4. To develop methods for semen collection, sperm storage and artificial insemination in rhesus monkeys.

Methods Employed:

1. Monkeys are housed under carefully controlled conditions and maintained on specific hormonal treatment.
2. Surgical procedures include gonadectomy and hypophysectomy.
3. Standard biochemical procedures have been modified for use in urinary estrogen determinations.
4. Semen is collected by electro-ejaculation and either stored in liquid nitrogen or used for artificial insemination. Semen is examined for sperm concentration and motility.

Major Findings:

Studies on urinary gonadotropin excretion in rhesus monkeys have been continued. Serum gonadotropin and urinary gonadotropin levels from 136 pregnancies have been determined by the mouse uterine weight assay method. This method is sensitive at a dose equivalent to 1.0 International Unit of human chorionic gonadotropin.

Monkey chorionic gonadotropin (MCG) was found at day 15 in all pregnancy sera examined. After day 35, gonadotropin was not detectable. Peak serum levels of monkey chorionic gonadotropin were observed at days 20-25 after mating.

Morphological studies on the corpus luteum of pregnancy were also carried out. Luteal cells degenerated progressively at the same time that chorionic gonadotropin was produced.

Maximum serum levels of MCG at 335 I.U./100 ml were attained early in pregnancy. Urinary levels in excess of 200 I.U./24 hours were observed during this time (Publication No. 1). Urinary chorionic gonadotropin extracts have been prepared and dose-response relationships have been determined in the mouse uterine weight bioassay procedure.

The female rhesus monkey with its 28-30 day menstrual cycle, production of chorionic gonadotropin and morphologic similarity of reproductive system to that of humans should provide an excellent system for experiments in reproductive research. There have been no systematic studies on normal hormonal patterns with which we can compare results obtained from pharmacological or physiological experimentation in these animals.

Further studies have been concerned with biochemical methods for determination of normal patterns of estrogen excretion throughout pregnancy as well as examining the effects of adrenocorticotropin treatment on estrogen excretion in ovariectomized monkeys.

This has included: an evaluation of acid hydrolysis versus enzymatic hydrolysis of urine; development of methods for formation of derivatives to determine those best adaptable to gas-liquid chromatographic procedures; evaluation of thin-layer chromatographic solvent systems to be used in purification of estrogens before and after derivative formation; and the use of radioactive tracers to estimate recovery of the urinary estrogens.

Estrone, estradiol and estriol have been identified in pregnant monkey urine indicating the presence of steroid biosynthetic pathways similar to those of man. However, estriol is not greatly elevated near term as in man.

Continued studies of the effects of adrenocorticotropin on ovariectomized adult female monkeys have confirmed earlier evidence for ACTH stimulation of estrogen production. Estrogen activity was indicated by repair of atrophic endometrial glands and stroma, perineal sex skin "reddening", increased uterine size, and withdrawal bleeding following cessation of ACTH treatment.

Cortisol treatment (5 to 10 mg. daily for 30 days) of ovariectomized monkeys did not alter the atrophic endometrium indicating that possible conversion of cortisol to an estrogenic metabolite did not occur under these conditions. Cortisol is the major secretory product of the adrenal cortex in the rhesus monkey (Publication No. 3).

In experiments on 12 pregnant monkeys (Macaca mulatta) bilaterally ovariectomized at 8 to 20 days following mating, pregnancy was not maintained. Earlier experiments had shown that bilateral ovariectomy after the 20th day of pregnancy permitted normal gestation. Progesterone treatment maintained pregnancy following ovariectomy at 12 days post-mating. These experiments demonstrate a requirement for ovarian hormones to maintain pregnancy before the 20th post-mating day in the rhesus monkey. The mean gestation period in our colony is 167 days.

Several aspects of deciduoma (maternal placenta) formation in the estrogen-progesterone treated, ovariectomized monkey (Macaca mulatta) were explored. Sutures passed through the uterine lumen of these hormonally conditioned monkeys will induce formation of decidual cells in the endometrial epithelium. In the rodent, it has been suggested that histamine release is responsible for decidual development.

Three series of four estrogen-progesterone treated castrate monkeys received intrauterine solutions (5-10 mgs. each) of histamine dihydrochloride. Deciduomata were not produced by this chemical procedure in any case, although deciduomata were induced in the endometrium of mechanically-traumatized controls. In addition, two animals were treated with a systemic histamine-releasing agent with negative results.

The effects of the presence of a foreign body on decidual formation have been explored. Plastic pins were placed in the uterine lumen of estrogen-primed ovariectomized monkeys. Mechanical trauma of the endometrium was initiated during subsequent progesterone treatment. When the foreign body was placed in the uterus at the beginning of progesterone treatment a decidual response occurred. In a recent series of four monkeys, the decidual reaction was not produced when the foreign body was inserted into the uterus during estrogen-priming. Thus, the time of foreign body introduction may prove to be important in deciduoma inhibition.

We have continued to develop methods of electro-ejaculation, artificial insemination, and sperm storage in the rhesus monkey. Electro-ejaculation, using an instrument fabricated by the NIH Instrument Section, is being used to provide semen for attempts at artificial insemination and development of techniques for liquid nitrogen storage. Results of insemination experiments have been negative. Diluted semen has been stored in liquid nitrogen for several weeks with 30% sperm survival.

Proposed Course of the Project:

It is expected to continue these studies with emphasis on determining similarities and differences between monkey chorionic gonadotropin and human chorionic gonadotropin. Collaborative experiments on comparison of dose-response relationships and immunologic cross-reaction between these chorionic gonadotropins are in progress.

Collaborative studies are underway with Dr. Amos Palmer, DBS, in which chorionic gonadotropin levels are being evaluated throughout pregnancy in Macaca speciosa, a much more docile species than Macaca mulatta.

We anticipate following the remaining studies along the lines indicated. Emphasis will be placed on the biological and biochemical factors underlying deciduoma induction, possible steroid biosynthetic properties of the tissue and methods of inhibiting its formation.

Significance to Bio-Medical Research and the Program of the Institute:

The rhesus monkey (Macaca mulatta) is an exceptional experimental tool for use in primate reproductive research. One of the major difficulties in using these animals has been a lack of information concerning hormonal levels in blood and urine. The systematic studies undertaken in this laboratory are an attempt to provide quantitative data both for the menstrual cycle and throughout gestation.

Collection and storage of semen will enable us to study sperm, its transport, and use in artificial insemination.

An understanding of the chemical and mechanical induction of the maternal placenta as well as its possible role in hormone production may help resolve problems of sterility and fertility.

Honors and Awards:

Dr. William W. Tullner: Invited to participate in conference on the use of rhesus monkeys in research on reproduction, San Juan, Puerto Rico, January 1966.

Publications:

1. Tullner, W. W. and Hertz, R.: Chorionic gonadotropin levels in the rhesus monkey during pregnancy. Endocrinology 78: 204-207, 1966.

2. Tullner, W. W. and Hertz, R.: Normal gestation and chorionic gonadotropin levels in the monkey after ovariectomy in early pregnancy. Endocrinology 78: (May) 1966.

3. Tullner, W. W.: Adrenal mediated endometrial stimulation and withdrawal bleeding in the ovariectomized monkey. Endocrinology (in press).

Serial No. HD-RP2
1. Laboratory of Biology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Endocrine Factors in Reproduction

Previous Serial No: None

Principal Investigator: Dr. William W. Tullner

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 8
Professional: ½
Other: 7½

Project Description:

Objectives:

1. To gain basic information on reproductive processes in laboratory animals.
2. To investigate the hormonal interrelationships of the anterior pituitary-adrenal-reproductive systems.

Methods Employed:

Normal animals on standard diets were used for tumor transplantation, surgical procedures and assessment of pharmacologic activity of selected compounds.

Surgical procedures for gonadectomy, hypophysectomy and adrenalectomy were used.

Major Findings:

A. Adrenal-Mediated Androgenic Activity of Pituitary Tumors.

Earlier experimental findings in castrated male rats, carrying a transplantable pituitary tumor, have been extended. The tumor produces adrenocorticotropin, growth hormone and prolactin. Characteristic androgenic effects (prostate and seminal vesicle weight increase, repair of atrophic prostatic epithelium) were observed. After bilateral adrenalectomy, androgenic action was not found.

In hypophysectomized-castrated male rats, the pituitary hormones secreted by the tumor produced adrenal weight increases concomitant with producing androgenic effects on prostate and seminal vesicles.

The anti-gonadotrophic action of prolactin reported in male Ring Doves (Bates and Riddle, 1935) was observed in intact rats bearing this pituitary tumor. Testes, prostate and seminal vesicles were atrophied. This effect of prolactin has not previously been reported for male mammals. Our findings may reflect the high, circulating level of prolactin from these mammotrophic tumors.

B. Studies on Mechanical and Chemical Induction of Deciduomata in Rats

A standard procedure for deciduoma induction was established for ovariectomized rats. Estrogen-priming, followed by eight days progesterone treatment, with needle scratch trauma of the endometrium on day 4 consistently produced deciduomata as determined by uterine horn weight increase and histological evidence of decidual cell development. Histamine has been proposed as the inciting agent for decidual formation (Shelesnyak). The findings of the latter were confirmed. However, it was found that either physiologic saline solution or deionized water would produce similar decidual reactions when instilled into the uterine horn of a suitably prepared rat.

Insertion of a small piece of polyethylene tubing into one uterine horn of an estrogen-primed rat inhibited the decidual response to needle scratch in that horn but not in the opposite horn. Thus, it appears that the foreign body effect is a local one and does not extend to the opposite horn. This finding is in contrast to results obtained in cows and ewes (Hawks, Beltsville, Md.) in which the presence of a foreign body in one cornua of the bicornuate uterus will prevent decidual cell development in the opposite horn.

Proposed Course of the Project:

It is expected that we shall continue intensive investigation of the decidual reaction in rodents including induction and inhibition.

Further studies on the effect of embryonal hormones on the mammalian reproductive tract are underway. Our previous work demonstrated the capacity of the fetal rabbit testis to synthesize testosterone from steroid precursors. Currently, we are collecting large numbers of fetal pig gonads at various stages in an attempt to isolate androgens from the fetal gonad and to learn why testosterone alone does not cause mullerian duct regression when implanted during the indifferent stage of reproductive development.

Significance to Bio-Medical Research and the Program of the Institute:

Adrenocortical tumors or cortical hyperplasia in man induce androgen production in some cases. Results obtained with pituitary mammotrophic tumor of the rat demonstrate that androgens are produced as a consequence of excessive stimulation of the adrenal cortex by anterior pituitary tumor hormones.

We have previously reported similar effects produced by combinations of exogenous ACTH and prolactin in the orchiectomized male rat. These findings yield further evidence for effects of adrenal androgens on the accessory glands of the male reproductive tract as well as providing an insight into the pituitary hormones response for the elaboration of adrenal androgens.

Current experiments on deciduoma induction in rats indicate that factors other than histamine are involved in producing this endocrine-mediated response. Introduction of deionized water into the uterine lumen produces a similar response and raises the question as to the nature of the inducing stimulus.

An understanding of the mode of action of intrauterine devices is an unsolved problem in reproduction research. Although rapid tubal passage of ova has been proposed as an explanation for the effectiveness of these foreign bodies (Mastroianni), studies such as those presented here may provide evidence that inhibition of the decidual response is the reason for lack of blastocyst implantation (Hertz).

Honors and Awards:

1. Lecturer in Endocrine Chemistry, Georgetown University Graduate School, Washington, D.C., 1960 to present.

2. Lecturer in Endocrine Pharmacology, Walter Reed Otolaryngology, Basic Science Course, April 1966.

3. Invited to participate in Laurentian Hormone Conference, September 1963.

Publications: None

Serial No. HD-RP3
1. Laboratory of Biology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Study of Biotin Metabolism in Development and in Various Endocrinologic States

Previous Serial Number: None

Principal Investigators: Dr. Sidney Siegel, Dr. Roy Hertz

Other Investigators: Mr. Wilbert Nixon, Dr. Stephen Potkay

Cooperating Units: Animal Hospital Section, LAB

Man Years:
Total: 2
Professional: 2
Others: 0

Project Description:

Objectives:

Biotin metabolism is to be studied in embryonic and postpartum animal systems by comparing fetal and maternal levels of biotin. The accumulation of the completing cofactor for biotinyl enzymes can be used as a tool to study the biochemistry of development as related to both fatty acid metabolism and morphologic indices in the genital tract of several species as effected by normal and artificially altered endocrinologic states.

Methods Employed:

Biotin and its vitamers were assayed by two microbiological systems (Saccharomyces cerevasiae and Lactobacillus arabinosis). Hydrolytic procedures necessary to free biotin from tissue must be followed by such purification techniques as paper chromatography, thin-layer chromatography, and sephadex column chromatography in order to establish identity of its metabolites. Tritiated biotin was assayed by converting the biotin present in organic material to tritiated water by the Schöniger flask combustion technique and counting the physical activity by liquid scintillation methods.

Major Findings:

A. Dogs

Comparison has been made in six pregnant dogs of maternal to fetal tissue levels of biotin and/or its derivatives as measured by microbiological methods and the ability to incorporate the labeled vitamin by various tissues. These animals were between 0.8 - 0.9 term.

The above three assay techniques were used to monitor the changing concentration of biotin and/or its vitamers in ten maternal compared to postpartum pup sets, from day +1 to +18 of life.

Concentration in Prenatal and Maternal Tissues:

a) Transplacental movement of the labeled material was established with the fetal tissues incorporating biotin at least at the level of matching maternal tissue.

b) Heart: no difference between maternal and fetal stable or labeled biotin concentrations.

c) Kidney: fetal and maternal incorporation of tritiated biotin was at the same level. Maternal stable biotin concentration 50% higher than unborn pups.

d) Liver: maternal levels, compared to their fetuses elevated for all three determinations.

e) Brain: fetal microbiologic and tritium concentrations 2-10 times higher than maternal tissue.

f) Muscle: demonstrates the most remarkable difference between mother and fetus. Fetal concentration of the stable biotin assayed by Saccharomyces 7-35 times and by Lactobacillus 11-80 times higher than mother muscle. Tritiated biotin activity 3-75 times higher in fetal muscle.

Concentrations in Postnatal and Maternal Tissues:

a) Heart: pups and mothers had biotin assay levels which were similar up to 18 days of life.

b) Kidney: maternal concentrations of stable biotin elevated compared to their pups for as long as the animals were followed. Pup ability to incorporate tritiated biotin matched mother level after four days.

c) Liver: pup concentration of Saccharomyces, Lactobacillus and ^{3}H biotin reached maternal levels after four days.

d) Brain: newborn levels for microbiologic and labeled biotin remained higher than maternal tissue for five days postpartum.

e) Muscle: the differences found in prenatal tissue by the three determinations is reduced toward adult levels by the third and fourth days of life.

B. Rabbits:

Maternal-fetal biotin concentrating and incorporating capabilities were studied in New Zealand white rabbits 90% term. A pattern of maternal-fetal differences not as gross, but similar to the dog was found.

C. Genital Tract:

Studies preliminary in nature have been carried out to determine if an altered endocrinologic state (natural or artificial) has an effect on various anatomic segments of the genital tracts of chickens and rabbits.

D. Human:

A total of 25 clinical studies in both male and female adult subjects were carried out. Tracer doses (3.5λ/4.0μc) of biotin H^3 were administered orally, intravenously, and intramuscularly. The urinary excretion of the physical activity followed. The route of administration did not affect the pattern of tritium excretion. Forty to eighty percent appeared in the urine in the first 24 hours, 1-4% the second 24 hours, 0.5 -2.0% the third day.

A change in the specific activity of the tritiated biotin has been noted. The change documented to be the result of:

1) Physical activity decay
2) Back exchange into the storage solvent
3) The slow appearance of a labeled vitamer of biotin (probably biotin sulfone or biotin sulfoxide)

Column chromatographic techniques have allowed proper purification to be carried out when authentic biotin is needed.

Significance to Bio-Medical Research and the Program of the Institute:

Studies in the area of prebiotic chemical evolution purport to show that among the most primitive of the enzyme systems are the ones needing folic acid and biotin for completion. The pluripotent nature of cells during the early stages of development has been well established.

The ability of certain embryonic tissues to accumulate high levels of a cofactor cannot be explained on the basis of: 1) overall growth rate differences between fetus and adult, 2) differences in activity of the characterized biotinyl enzymes-fetus compared to adult.

Morphologic changes occurring during prenatal and postnatal development have been recorded for many species. The appearance and accumulation of a cofactor not only specific for certain enzymes but also assayed and/or

isotopically labeled with relative ease offers a tool for logical study in quantitating the biochemistry of normal and pathological development.

Specifically the accumulation of a cofactor can be used to monitor when a "gene is turned on" for the elaboration of a specific protein. It might then become possible to correlate morphological with biochemical changes during the various phases of prenatal and postnatal development. Biochemical changes which could be monitored with isotopically labeled cofactor are: 1) determining the presence or absence of an enzyme or group of similarly dependent enzymes, 2) reaction rate changes during development of an enzyme or group of related enzymes, 3) isotopes such as C^{14}, S^{35}, P^{32}, Co^{60} could be used to label essential cofactors and enable studies concerning their metabolism to be run in parallel in the same experimental animal.

Proposed Course of the Project:

1. Extend the findings in the dog and rabbit on biotin accumulation and concentration to other species, chicken, monkey and man. This would include going to earlier stages of development in any system studied.

2. Development of procedures for the concentration, extraction and characterization of biotin and related metabolites from tissue and urine.

3. Study the effect on cofactor metabolism in various anatomic segments of the genital tract (in normal and artifically altered endocrinologic states) of several mammals in order to determine whether there is an area of localized high biotin activity such as that found in the chick oviduct.

Honors and Awards:

1. Invited Lecturer. Eighth annual Beta-Sigma Chapter of Rho Chi National Honor Society (Howard University Chapter), December 1965.

2. Invited Lecturer. Graduate School of Arts and Sciences; Catholic University, Biology Department, March 1966.

Publications:

None

NICHD ANNUAL REPORT
July 1, 1965 through June 30, 1966
Endocrinology and Metabolism Branch

SUMMARY

The staff of the clinical Endocrinology and Metabolism Branch, consisting of two senior physicians, four clinical associates, two biochemists, four technicians and one secretary have united as an effective team for the practice of clinical medicine and the conduct of clinical and laboratory research. Efforts begun by this group while affiliated with the Endocrinology Branch of NCI have been continued, largely without interruption.

Areas of investigative interest include the following:

1) Biological and radioimmunological assays of protein and polypeptide hormones

2) Isolation and purification of protein and polypeptide hormones

3) Cytogenetic aspects of endocrine disorders

4) Hormonal induction of ovulation

5) Follow-up of patients successfully treated for gestational trophoblastic neoplasms

6) Application of computer technology to problems in clinical and laboratory investigation

The inter-relations of our individual and joint efforts will be apparent from the discussion of results obtained as the several interest areas are considered in detail. This integration is the spontaneous derivative of a community of interests rather than the result of administratively-imposed, goal-oriented or directed research.

Serial No. HD-RP4
1. Endocrinology and Metabolism Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Assays of Protein and Polypeptide Hormones in Biologic Fluids

Previous Serial Number: NONE

Principal Investigator: Dr. William D. Odell

Other Investigators: Dr. Griff T. Ross, Mr. Phillip Rayford, Dr. Richard Gardner, Miss Elizabeth Nourse, Mr. Robert Wright, Dr. Howard Kulin, and Dr. Arleen Rifkind

Cooperating Units: Dr. Mortimer B. Lipsett, Dr. Peter Kohler, Dr. Bert O'Malley, Dr. Wayne Bardin, Dr. John Wilber, Endocrinology Branch, NCI; Radiopharmaceutical Service, NCI

Man Years:
Total: 5
Professional: 5
Other:

Project Description:

Objectives: 1. Development of methods sufficiently sensitive to enable the measurement of some protein and polypeptide hormones in blood and urine of man and experimental animals.

2. The application of these methods to the study of factors influencing the levels of these hormones in blood and urine in normal and pathophysiologic states.

Methods Employed: The methods of radioimmunoassay, coupled with biological assay, where applicable, have been utilized. Immunoassay methods have been developed in this laboratory for human thyrotropin (TSH), human luteinizing hormone (HLH), and alpha melanocyte-stimulating hormone (MSH). A method for the assay of human growth hormone (HGH) developed elsewhere has been adapted for use in this laboratory.

Major Findings:

A. Thyroid-stimulating Hormone (TSH)

Estimations of secretion rates of TSH in euthyroid and hypothyroid human subjects have been completed. Reasonably reliable estimates of degradation time and secretion rates of TSH in man have been made for the

Major Findings (cont'd)

first time. Results show that in hypothyroidism, secretion rates are increased and degradation time prolonged, accounting for the high levels of TSH observed in the plasma of patients with primary myxedema.

Similar studies have been performed in cattle using a radioimmunoassay for bovine TSH.

B. Luteinizing Hormone (LH)

A radioimmunoassay for luteinizing hormone in man (HLH) has been developed. The method is capable of detecting and quantifying this hormone in volumes of 0.3 ml. of plasma or less and is about 500 times as sensitive as the most sensitive biologic assays.

For the first time levels of luteinizing hormone have been followed in plasma of normal women daily throughout the menstrual cycle. A single sharp peak of activity has been found, occurring about mid-cycle, lasting for 24 hours, and associated with presumptive evidence of ovulation (elevation of basal body temperature and increased urinary pregnanediol excretion). The height of these peaks equals or exceeds levels regularly found in post-menopausal women. In women with ovaries and irregular menses, minor day-to-day variations have been observed for periods of up to 50 days of amenorrhea. Levels in normal males show minor daily variations and no significant variation has been shown diurnally or in relationship to bed rest or ambulation. In contrast, levels are high in castrate males.

Clomiphene, an agent used in attempts to induce ovulation in anovulatory or oligo-ovulatory women, has been shown to cause an elevation of plasma luteinizing hormone in both men and women. Studies are currently under way to determine the effects of the oral contraceptive agents on plasma luteinizing hormone activity.

Parallel studies, including immunoassays of blood and biologic and immunologic assays in urine, are being carried out in a population of prepubertal children ranging in age from 6 to 9 years. These investigations, in addition to providing information of clinical usefulness in evaluating urinary gonadotropin excretion, should provide some information about the hormonal changes incident to puberty.

The assay methods offer promise in the clinical evaluation of disorders of pituitary and gonadal function.

C. Melanocyte-stimulating Hormone (MSH)

Considerable progress has been made in the development of a radioimmunoassay for alpha melanocyte-stimulating hormone. The assay can be carried out with excellent sensitivity and precision in the absence of serum. So

Major Findings (cont'd)

far, it has not been possible to demonstrate any alpha MSH in either blood or pituitary tissue of humans but it appears to be present in pituitary tissue of albino rats. The method is not sufficiently sensitive to permit detection of this hormone in the blood of normal rats.

The half-time of disappearance of alpha MSH injected into hypophysectomized rats has been studied by three methods:

1. Following disappearance by biologic assay using hypophysectomized frogs
2. Following disappearance by radioimmunoassay
3. Following disappearance of tracer amounts of radioactively labeled alpha MSH

Data obtained thus far show a half-life of 1.6 minutes by bioassay and of 2.1 minutes by radioimmunoassay. A much longer half-life of 7.8 minutes has been observed in following the disappearance of the radioactively labeled hormone. Studies of the basis for this disparity indicate that the process of labeling alpha MSH results in alterations in the molecule so that biologically it is handled differently than unlabeled hormone. This important observation has considerable pertinence to studies using tracer amounts of isotopically labeled hormones in determination of plasma half-life, secretion rates, etc.

D. Growth Hormone

A clinical test has been devised for the evaluation of pituitary growth hormone secretory capacity by measuring plasma levels of this hormone before and after the administration of pyrogens. The test has the advantage that pituitary ACTH reserve can also be tested simultaneously by measurement of plasma cortisol levels. This test has been utilized in evaluating patients suspected of having pituitary dysfunction in order to ascertain whether the dysfunction relates to one or more pituitary hormones.

Proposed Course of the Project: These methods will be applied to the further elucidation of factors influencing hormone levels in normal and pathophysiologic states in man. The importance of these studies in the area of human reproduction hardly requires further elaboration. Extensive efforts will be directed toward development of similar assay methods for other protein and polypeptide hormones.

Significance to Bio-medical Research: For the first time assays are available that are capable of quantifying these hormones in volumes of blood that can be obtained daily (or several times a day if necessary). Further, levels can be appreciated in the blood of normal patients in most instances so that pathophysiologic states relating to both increased and decreased secretion may be identified.

Significance to Bio-medical Research (cont'd)

With respect to reproduction, the physiologic basis for the use of agents capable of suppressing ovulation can be studied. Alternative methods can be compared. Further, studies in human infertility are possible.

The study of the developmental aspects of gonadal function provide a most interesting area for future study.

Finally, the availability of a variety of assay methods facilitates the study of hormonal interactions in normal and pathophysiologic states.

Honors and Awards (Dr. William D. Odell)

Lecturer at Baylor University Hospital, Dallas, Texas, August, 1965.

Lecturer at the University of Toronto, August, 1965.

Lecturer at D. C. General Hospital, Georgetown Medical Service, Medical Grand Rounds, September, 1965.

Lecturer at University of California at Los Angeles, February, 1966, and Harbor General Hospital, Torrance, California.

Speaker at Georgetown University School of Medicine, March, 1966.

Series of 12 lectures at George Washington University School of Medicine, Department of Physiology, April, 1966.

Presented paper at annual meeting of American College of Physicians, New York City, April, 1966.

Will present paper at annual meeting of Society for Clinical Investigation, Atlantic City, May, 1966.

Associate Professorial Lecturer, 1965-66, George Washington University School of Medicine, Department of Physiology.

Consultant in Research, Graduate Council, George Washington University School of Medicine, 1966-67.

Instructor, Elective course lasting one semester, series of 24 lectures, George Washington University School of Medicine, September 1965 to January 1966.

Honors and Awards (Dr. Griff T. Ross)

Speaker, Thursday Evening Grand Rounds, NIH, January, 1966.

Honors and Awards (Dr. Griff T. Ross)

Lecturer, Medical Grand Rounds, Georgetown University Service, D. C. General Hospital, April, 1966.

Will lecture at biennial meeting of the Hospital de Gineco-Obstetricia No. Uno, Instituto Mexicano Del Seguro Social, Mexico City, May, 1966.

Publications

1. Odell, W. D., Ross, G. T. and Rayford, P. L.: Radioimmunoassay for human luteinizing hormone. Metabolism 15: 287, April 1966.

2. Wilber, J. F., Odell, W. D., and Kiddy, C.: A Radioimmunoassay for Serum TSH in the Cow. Accepted for publication in Endocrinology.

3. Odell, W. D., Swain, R. W., Bowers, C. Y. and Schally, A. V.: Radiation Inactivation of Purified Lysine Vasopressin. Accepted for publication in Biochim. et Biophys. Acta.

4. Odell, W. D.: Isolated Deficiencies of Anterior Pituitary Hormones. Accepted for publication in J.A.M.A.

5. Wilber, J. F. and Odell, W. D.: Influence of Tapazole upon Serum TSH. J.C.E.M. 25: 1407, 1965.

6. Ross, G. T., Odell, W. D., and Hertz, R.: Diagnostic Implications of Primary Amenorrhea. Accepted for publication in Ann. Int. Med.

Serial No. HD-RP5
1. Endocrinology and Metabolism Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Cytogenetic Aspects of Endocrine Disorders

Previous Serial Number: NONE

Principal Investigators: Dr. Griff T. Ross, Miss Elizabeth Nourse

Other Investigators: None

Cooperating Units: Dr. J. H. Tjio, Laboratory of Experimental Pathology, NIAMD; Drs. John Marshall and James Holland, Surgery Branch, NCI; Dr. Robert Henkin, Clinical Endocrinology Branch, NHI; Dr. Jean Herdt, Diagnostic Radiology Department, CC; and Dr. Saul Rosen, Clinical Endocrinology Branch, NIAMD

Man Years:
Total: 4½
Professional: 4½
Other:

Project Description:

Objectives: A collaborative, multidisciplinary study of the cytogenetic aspects of disorders of gonadal development and function.

Methods Employed:

1. Classical techniques for the study of chromosome number and morphology in blood, bone marrow and other tissues, particularly gonadal tissue (Drs. Ross, Tjio, Marshall and Holland)

2. Dermatoglyphics (Dr. Marshall)

3. Roentgenographic evaluation of osseous malformations (Dr. Herdt)

4. Studies of taste and smell by techniques developed by Dr. Henkin

5. Visualization and biopsy of gonads by culdoscopy in women and direct surgical exploration in men (Dr. Marshall)

6. Biologic and immunologic assay of protein hormones in blood and urine (Drs. Ross and Rosen)

Major Findings: This study in its present format has only recently been undertaken. Activities thus far have been confined to attempts to standardize and develop methods of study. All of the physicians listed see patients who present with problems of development and function of gonads. By joint consultative efforts, each investigator brings his skills to bear on the individual patient, so that thorough evaluation is possible and each investigator's experience is increased.

The feasibility of a culdoscopic biopsy of gonadal tissue for chromosomal studies has been demonstrated in two patients, both of whom had small malformed ovaries--the one undifferentiated streak gonads, the other small hypoplastic ovaries secondary to hypogonadotropic hypogonadism. It is thought that chromosomal abnormalities limited to gonadal tissue may be responsible for failure of differentiation in some instances.

Studies of dermatoglyphics, osseous malformations, and taste and smell may further our understanding of the modes of inheritance of extragenital stigmata associated with hypogonadism.

Significance to Bio-medical Research: Studies of cytogenetic correlations in patients with disorders of gonadal development and function have largely failed to be satisfactory to date. This relates to inadequate evaluation in many instances.

The combination of the skills of a number of investigators, which is virtually unique at the NIH, makes possible exhaustive studies of the relationships between chromosomal abnormalities and disorders of gonadal development and function.

Proposed Course of the Project: Continued collaborative efforts in the area described.

Honors and Awards (Dr. Griff T. Ross)

Lecturer, Department of Physiology, George Washington University School of Medicine, November 1965.

Lecturer and participant in Cytogenetics Workshop, Applied Seminar of the Association of Clinical Scientists, Washington Hospital Center, November 1965.

Appointed member of Medical Board, January 1966.

Publications

1. Ross, G. T. and Tjio, J. H.: Cytogenetics in Clinical Endocrinology of Infancy and Childhood. Clinical Pathology of Infancy. Accepted for publication in Proceedings of an Applied Seminar of the Association of Clinical Scientists, Sunderman & Sunderman, Eds.

1. Endocrinology and Metabolism Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Determination of Minimal Ovulatory Dose of Human Menopausal Gonadotrophins (HMG) in HMG/Human Chorionic Gonadotropin (HCG) Ovulation Induction

Previous Serial Number: NONE

Principal Investigator: Dr. Charles B. Hammond

Other Investigator: Dr. John R. Marshall

Cooperating Units: Surgery Branch, NCI

Man Years

Total: 1
Professional: 1

Project Description:

Objectives:

To determine the minimal ovulatory dose of HMG when used in combination with HCG for the induction of ovulation in the human female.

Methods Employed:

1) Complete diagnostic evaluation using standard clinical and laboratory studies.

2) Measurement of response to test dose of HMG.

3) Sequential injection of increasing doses of HMG days 1, 4 or 8 and 1200 I.U. HCG on day 10.

4) Response measured in terms of the following:

a) Estrogen effects: vaginal cytology, cervical mucous

b) Ovulation: basal body temperature, urinary pregnanediol excretion, endometrial biopsies on day 1 of bleeding.

Major Findings:

Twenty-five patients are currently under therapy with 10 others being evaluated diagnostically for possible therapy. The population will be increased as time permits.

Results indicate a sigmoid dose response relationship with incidence of ovulation increasing with increasing doses of HMG. Minimal ovulatory doses range from 450 to 1725 I.U. of HMG. Grouping of patients by diagnosis results in a marked grouping of the minimal ovulatory doses. Present findings would suggest that the generally accepted and widely used dosage schedule of daily HMG injections is excessive in most instances and that smaller divided doses could be used with equally satisfactory, if not more satisfactory results.

Significance to Bio-Medical Research:

Human menopausal gonadotropin (Pergonal®) promises to be a useful agent in the treatment of anovulation in women desiring conception. The present problems associated with this drug are such as to preclude its widespread and unregulated use without inordinate hazard relating to superovulation, development of ovarian cytomas, etc. It is our feeling that these unwanted side effects are largely dose related and could be eliminated by more appropriate selection of dosage. Information derived from this study will test this hypothesis and hopefully provide a basis for valid selection of dosage for the individual patient.

Ancillary Studies:

Ancillary studies which are under way as part of the Pergonal study include the measurement of human chorionic gonadotropin in patients with Pergonal-induced pregnancies; the early detection of plasma chorionic gonadotropin in the first two weeks of gestation; determination of anti-Pergonal antibody in patients treated with Pergonal; determination of the effects of Pergonal on plasma testosterone and androstenedione levels; the development of an "ovarian stimulation test" in which patients are categorized by their production of estrogen as measured in urine in response to a standard dosage of Pergonal and the measurement of plasma FSH/LH levels in anovulatory patients prior to Pergonal therapy.

Proposed Course of Project:

The project is continuing with the additional succession of patients.

Honors and Awards: None.

Publications: Marshall, J. R., and Hammond, C. B.: Ovarian biopsy under culdoscopic visualization. Accepted for publication in Am. J. Obst. and Gynec.

Serial No. HD-RP7
1. Endocrinology and Metabolism Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Isolation and Purification of Protein and Polypeptide Hormones

Previous Serial Number: NONE

Principal Investigator: Dr. John Cooper

Other Investigators: Dr. W. D. Odell, Dr. Griff T. Ross, Mr. Frederick Dhyse

Cooperating Units: Dr. Peter Condliffe, Laboratory of Nutrition and Endocrinology, NIAMD
Dr. Robert Bates, Laboratory of Nutrition and Endocrinology, NIAMD
American Pituitary Foundation

Man Years

Total: 4
Professional: 4

Project Description:

Objectives:

1. The procurement of highly purified protein and polypeptide hormones in quantities sufficient for physiologic studies.

2. Purification of protein and polypeptide hormones for use in radioimmunoassay procedures.

Methods Employed:

1. Standard methods for extraction and separation of proteins including

   a. Differential solubility
   b. Gel filtration
   c. Disc gel electrophoresis
   d. Ion exchange chromatography.

2. Biologic and immunologic assay techniques for monitoring purification.

Major Findings:

A. Chorionic gonadotropin

Pooled kaolin-acetone extracts of urine of women with choriocarcinoma have been purified by ethanolic fractionation. Gonadotropin preparations with specific activities in excess of 10,000 I.U. per mg. have been obtained. Isotopic labelling and reaction of this material with antibodies raised to human chorionic gonadotropin indicates that it is still not sufficiently pure to be useful in radioimmunoassays.

Inadequate information as to the stability of highly purified preparations of chorionic gonadotropin has prevented further batch purification activities. It is anticipated that further purification can be achieved by ion exchange chromatography and preparative disc gel electrophoresis.

B. Human pituitary hormones

Ten thousand human pituitary glands have been obtained from the American Pituitary Foundation. This material has been extracted by a modification of the percolation procedure of Bates. Bioassays indicate efficient extraction of TSH, gonadotropins and growth hormone. Initially, separation of growth hormone from the other hormones will be attempted. Then separation of TSH and gonadotropins will be attempted. This material should provide ample quantities of gonadotropins and TSH for extension of clinical studies described previously.

Biomedical Significance:

A paucity of highly purified preparations has been one of the factors limiting studies of endocrine physiology as it relates to protein and polypeptide hormones in man. Highly purified preparations can be utilized as 1) antigens for the generation of hormone specific antisera, 2) antigens for isotopic labelling for studies of secretion rates, production rates, metabolic clearance rates, etc. It is hoped that sufficient material will be obtained not only for our own use but for the use of other investigators in this field.

Projected Course of the Study:

Continuation of the project as outlined.

Honors and Awards: None

Publications: None

Serial No. HD-RP8
1. Endocrinology and Metabolism Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Application of Computer Technology to Problems in Clinical and Laboratory Investigation. A. The Statistical Analysis of Bioassay Data by Digital Computer Techniques.

Previous Serial Number: NONE

Principal Investigator: Dr. J. A. Cooper, III

Other Investigators: None

Cooperating Units: Division of Computer Research and Technology

Man Years

Total: 1/2
Professional: 1/2

Project Description:

Objectives:

Efficient statistical evaluation of results of biologic assays.

Methods Employed:

1) Standard statistical procedures Fortran IV, E level subset program for IBM operating system/360.

Results:

A basic statistical routine designed for use with the IBM 407 operating system was rewritten with extensive modification in Fortran IV, E level subset for the IBM operating system/360. The program has now been catalogued and is being run on a production basis. Regression analysis by least square methods together with analysis of variance and covariance are now available on a routine basis. The usefulness of this facility in evaluating the results of biologic assays is immeasurable. The time required when such an analysis has to be done with a desk-type calculator is about 8-10 hours for a large assay. One to two hours for data transcription is all that is required with the aid of this system.

Biomedical Significance:

Statistical validation of the results of biologic assays is mandatory for interpretation of findings and particularly important in the matter of communicating results in terms of standard preparations. When the data are treated in this manner, results in different laboratories can be compared.

Projected Course:

Methods developed will be applied in the assays utilized in following the course of purification of the hormone preparations discussed before. Studies of alternative methods of assay are currently being made.

Honors and Awards: None

Publications: None

Serial No. HD-RP9
1. Endocrinology and Metabolism Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Computer Analysis of Clinical Data in Patients with Malignant Gestational Trophoblastic Neoplasms.

Previous Serial Number: NONE

Principal Investigator: Dr. Charles B. Hammond

Other Investigators: Dr. Griff T. Ross, Mr. Arnold S. Pratt, Mr. William White

Cooperating Units: Computer Section, NCI

Man Years

Total: 1 1/4
Professional: 1 1/4

Project Description:

Any detailed manual analysis of the very long and complex clinical and laboratory records of the 196 patients with gestational trophoblastic neoplasms (GTN) is very difficult. If done by hand, only a few factors, judged a priori to be of significance, can be obtained, even with the efforts of several persons for long hours. Many other factors influencing the effects of chemotherapy cannot be determined.

Objectives:

Detailed computer analysis of the clinical courses, response to chemotherapy and outcome of 196 patients with malignant gestational trophoblastic neoplasms, treated by physicians of the Endocrinology Branch, National Cancer Institute during the 10 year span from 1956 to 1966.

We have designed a method for total chart abstraction--without significant data prejudice by the abstractor--keying every entry upon date and code word heading for the appropriate observation (clinical or laboratory). Some 160 of the 196 charts have been so abstracted.

Work is currently being done in conjunction with the Computer Division of NCI to enter these data into a IBM 1620 computer. The voluminous data files are being processed for loading by both flexowriter and keypunch entry systems. Fifty percent of these data have now been loaded and it is anticipated that this phase will be completed by June 1, 1966.

Upon completion of loading and programming retrieval, these files will be repeatedly and systematically reviewed in the computer to allow detailed analysis of any and all factors influencing these patients' courses and responses to chemotherapy. In addition, the computer files of the Department of Pathologic Anatomy, NIH, will be cross-linked to allow study of the clinical pathological correlations of patients dying from these diseases.

Major Findings:

Data abstraction and loading have comprised the bulk of effort to date. Preliminary retrieval runs have been carried out and demonstrate the functional effectiveness of the system. Full analysis of the entire series will begin shortly. It is apparent that this system offers an excellent opportunity for detailed analysis of complex clinical records.

Significance to Bio-Medical Research:

Such a system as described above offers the first efficient method for detailed analysis of any clinical medical record. In addition, it will allow, for the first time, an accurate method of studying the complex constellation of clinical response to chemotherapy by patients with gestational trophoblastic neoplasms.

We feel this system can be easily and efficiently altered to process other types of clinical data.

Proposed Course of the Project:

Loading and search of data files is continuing. It is anticipated that retrieval will be carried out for what is now an undefined span of time.

Honors and Awards: None

Publications: None

Serial No. HD-RP10
1. Endocrinology and Metabolism Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Follow-up of Women Treated Successfully with Chemotherapy for Gestational Trophoblastic Neoplasms

Previous Serial Number: NONE

Principal Investigators: Dr. Roy Hertz and Dr. Griff T. Ross

Other Investigators: Dr. Charles B. Hammond

Cooperating Units: NCI Tumor Registry

Man Years:
Total: 1
Professional: 1
Other:

Project Description: More than 100 women are in remission following chemotherapy for gestational trophoblastic neoplasms. The agents used in treatment are known to be both oncogenic and teratogenic in animals, but no data are available in man. These patients represent a unique group for the study of the long-term effects of these agents on reproduction.

Objectives: Continued follow-up of these patients with respect to the following:

1. Continuation of remission status
2. Development of new neoplasms
3. Results of subsequent pregnancies

Methods Employed: Charts are reviewed at 6-monthly intervals for evidence of contact with either patient or referring physician. Urines are studied at intervals of 6 months to 1 year for gonadotropins. Periodically, patients are written to ascertain interval reproductive history.

Major Findings: Data are currently being reviewed for publications. Preliminary data do not indicate any unusual incidence of 1) early abortions, 2) stillbirths at term, or 3) congenital malformations in infants born at term.

Significance to Biomedical Research: As advances in chemotherapy of malignancy are made, it becomes important to have some data on the oncogenic and teratogenic potential of agents utilized. Since patients with choriocarcinoma and related neoplasms appear to have the greatest likelihood of

Significance to Biomedical Research (cont'd)

prolonged sustained remission following chemotherapy, this group of patients represents a unique opportunity for such studies.

Proposed Course of Project: Continuation of follow-up procedures with periodic review as to efficiency.

Honors and Awards (Dr. Griff T. Ross)

Blackford Memorial Lectures at Sherman, Texas, September 1965.

Speaker at Grand Rounds, Department of Obstetrics and Gynecology, Johns Hopkins University, October, 1965.

Lecture, Mount Sinai Hospital, New York, First Conference on Chemotherapy of Malignancies, November 1965.

Speaker and participant, First Workshop on Chemotherapy of Trophoblastic Neoplasms, Harvard Medical College, February, 1966.

To participate in roundtable discussion on Chemotherapy in Gynecologic Malignancy at meetings of American College of Obstetricians and Gynecologists, Chicago, May, 1966.

Publications

1. Ross, G. T., Goldstein, D. P., Hertz, R., Lipsett, M. B. and Odell, W. D.: Sequential use of Methotrexate and Actinomycin D in the treatment of metastatic choriocarcinoma and related trophoblastic diseases in women. Am. J. Obst. & Gynec. 93: 223 (Sept) 1965.

2. Ross, G. T., Hammond, C. B., Hertz, R., Lipsett, M. B. and Odell, W. D.: Chemotherapy of metastatic and non-metastatic gestational trophoblastic neoplasms. Accepted for publication in Texas Reports in Biology and Medicine.

NICHD Annual Report
July 1, 1965 through June 30, 1966
Growth and Development Program

The final approval of the organizational pattern for the entire Institute of Child Health and Human Development in December 1965 brought formal structure to the Growth and Development Program. This area represents one-fourth of the Institute's scientific program. One-third of the Institute's extramural activities are in the Growth and Development Program.

Program goals were developed by various methods during the year. Staff spoke to research groups on six university campuses. Thirteen additional presentations of scientific material or research goals, including organizing and moderating a symposium at the Annual Meeting of the Federation of American Societies for Experimental Biology, were made before national or regional professional organizations and groups.

In addition to bringing several distinguished American scientists to the Program for consultation, the staff arranged for Dr. Anna Freud and Dr. Hans Papousek to visit NIH and address those interested in their respective fields of research.

One member of the staff joined with a group of pediatric educators for a three-day meeting on Pediatric Education in Latin America sponsored by the Pan American Health Organization.

At the International Congress of Pediatrics the Program Director spoke to a group convened to discuss the broad aspects of growth and development. At the close of the meeting it was suggested that an informal organization of this international group be established to exchange information on the research publications of each member through the NICHD Growth and Development Program offices.

The document on the Behavioral Science interests of the Institute prepared for Council presentation in March 1965 appeared in the AMERICAN PSYCHOLOGIST in August 1965, the AMERICAN SOCIOLOGIST in February 1966, and was summarized in the AMERICAN ANTHROPOLOGIST. This has brought the broad range of interests of this Program to the attention of the behavioral scientists throughout the country.

Perhaps the most successful conference held by the Program since its inception was the joint meeting of fifteen outpatient pediatric clinic directors and fifteen leading behavioral scientists. The topic for discussion was Conceptual and Methodological Approaches to Research in Pediatric Outpatient Departments and was a most effective means of stimulating cross-disciplinary interaction.

In May 1966 the Program convened the Learning Advisory Group Meeting for a major conference on research in learning.

The Growth and Development staff is working with the Information Center staff in the final phases of the preparation of a working vocabulary for indexing the literature in this field. One working conference was held with several of the same consultants used a year ago.

The proceedings of the Conference on Sudden Death in Infants held in Seattle, Washington, September 1964, were published in May 1966. Distribution of the proceedings upon request to physicians, investigators, and organizations supporting research on this subject has been initiated.

The Institute staff has continued to make the MEDLARS sudden death bimonthly printout available to investigators working in the field.

The Growth and Development Program has assumed administrative responsibility for six of the Institute's clinical or research associates. Each physician has been placed with research laboratories outside NICHD. The activities are briefly summarized as follows:

(1) Clinical neurology combined with a research experience-study of various aspects of cortical maturation in the cat. Staining techniques for the immature cortex have been a major problem in the development of the research. One immediate goal is to quantify the pattern of dendritic growth of the cortical neurons.

(2) In immunochemistry - a study to elaborate and refine a new method to quantitatively estimate immune cytolysis of nucleated cells; differentiation of various murine leukemias by complement fixation and application of this test to studies of rabbit antibody to mouse leukemia; the ontogeny of sheep complement; the evaluation of complement in neonatal cord blood and amniotic fluid and correlation with respect to hemolytic disease of the newborn.

(3) In a biochemistry laboratory - a study of the biological properties of rodent mammary tumors of varying functional differentiation. Tumors have been produced in rats by injection with nitrosourea. The initial problem has been the development of methods for the quantitation of milk production by tumors.

(4) In surgery, clinical care of patients; preparation of a manuscript on hypernephromas; developing plans to study the effect of Dextran infusions in reducing the incidence of metastases.

(5) In oro-pharyngeal development - Took part in a review and analysis of dysphagia in infants; then initiated an anatomical, physiological, and orthodontic study which includes cineradiography. A spirometer has been adapted to measure palatal-pharyngeal insufficiency. Sucking pressure and sucking behavior is under study in cleft palate infants.

(6) In biochemistry - various mechanisms of growth, particularly recovery growth is under study in children with cardiac defects, endocrine abnormalities and malnutrition along with their normal siblings. Also, rats are being studied, observing the effect of starvation, hormonal deficiency and stress. Work on caloric expenditure and dietary intake under various conditions for children has been undertaken.

The extramural program supported a total of 448 grants amounting to $13,876,214 as of March 31, 1966. (Not all of the March Council actions had been activated by that date.) 73.9 per cent (331) of the grants were in direct research, 8.7 per cent (39) were in training, 10.3 per cent (46) were in fellowships, and 4.9 per cent (32) were in the career award program.

The extramural activity has three major subsections: (a) biological sciences, (b) behavioral sciences, and (c) training. Ongoing programming in each area is as outlined:

Biological sciences: Molecular biology including biochemistry of differentiation and neurochemistry, biochemical genetics, and endocrinology; developmental immunology; developmental physiology including physical growth; and nutrition.

Behavioral sciences: Physiological correlates of behavior; cognition and learning, social development; personality development; and psycholinguistics.

Training: In addition to the above areas where ongoing research will support training, the Program supports such additional training as the broad concepts of clinical research in pediatrics including pediatric radiology.

To aid in the extramural programming, one staff scientist (Ph.D.) has been added in each subsection. One M.D., formerly in the clinical associate program, will receive his M.P.H. in June and join the extramural staff at that time. Particular emphasis has been given to research in learning, nutrition, neurophysiology and neurochemistry.

At the close of the Program's first year of extramural activity the percentage of the grants in research (domestic and foreign), training, fellowships, and career awards was determined. There have been only minor variations in this distribution since that time with the biological science-behavioral science ratio remaining close to 60-40.

Contract Title: Study of relationship between Nutrition and Behavioral Development (PH43-65-640)
Contractor: Pan American Health Organization - Institute for Nutrition of Central America and Panama (INCAP). Dr. Cipriano Canosa.
Money Allocated: $160,000 (Continuation of FY 1965 funds)

This contract has been designed to contribute additional knowledge on the effect of nutrition on growth and development. The INCAP staff has had extensive experience in the study of nutrition, infection, and physical growth. Using isolated Indian villages similar to those used in the past, the specific aims are to explore the physical growth parameter of each child in the study from the newborn period to school age in order to determine (1) the state of nutrition and to study the interrelationship of the state of nutrition to infection, intellectual growth, and behavioral development, (2) at what age adequate nutritional supplements promote changes in physical growth, intellectual growth and behavioral development, and (3) the cultural and socio-economic factors that influence physical growth, intellectual growth and behavioral development at various ages.

After seven months of work in an Indian village by the contract staff, a group of biological and behavioral scientists from American universities joined the INCAP staff in the field for one week to discuss the most logical approach for the development of the instruments to be used in the psychological, sociological and anthropological fields.

Experimental design, food supplementation, data collection and processing were all discussed with a realistic evaluation of the problems before the research group.

Contract Title: A longitudinal and comparative study of cognitive development in the human infant. (PH43-65-1009)
Contractor: Harvard University. Dr. Jerome Kagan.
Money Allocated: $49,500 (Continuation of FY 1965 funds)

The primary intent of this entire project of three years is to inquire into selected aspects of cognitive and behavioral development during the first three years of life for children of differing socio-economic class backgrounds. Major goals are:

1. To determine if there is any link between individual differences in functioning during the child's first year and individual differences in cognitive structure and behavioral differentiation as revealed by language, perceptual functioning and play behavior of three-year olds.
2. To trace developmental aspects of the cognitive variables involved and examine relationships between attentional and other cognitive variables in infancy and childhood.

3. To study mother-child interactional patterns as a key variable in the understanding of some of the differences in the cognitive development of the two class groups.

Contract Title: Developmental trends in cognitive behavior. (PH43-65-1011)
Contractor: Fels Research Institute. Eugene S. Gollin
Money Allocated: $71,000 (Continuation of FY 1965 funds)

This research program was designed to study the development of cognitive behavior. The research program has been concerned with changes in intellective functioning in the course of development and with the identification of factors which accelerate or retard such changes. Conditions which facilitate the onset of transition and the training procedures which accelerate transition are under study. The children in the program range in age from approximately three years through thirteen years. Adult groups are employed for comparative purposes.

Contract Title: Survey of the Development of vocalization in infancy and early childhood. (PH43-65-637)
Contractor: The Johns Hopkins University, Neurocommunications Laboratory, Department of Psychiatry. Richard Allen Chase, M.D.
Money Allocated: $146,000 (Continuation of FY 1965 funds)

In the first year this contract was established to determine the earliest age at which vocalization activity in the human reflects distinctive features of the acoustic representation of adult speech; determine those elements in the catalog of early vocalization patterns that first demonstrate change in form, corresponding to features of the adult vocalization system; and determine those features of the adult vocalization system that are first represented in the speech vocalization of the infant.

Projected work is (a) to identify some of the critical acoustic features of the stimulus environment necessary for speech acquisition, (b) study the process by which the child learns to use his speech motor gestures meaningfully, (c) study the role of acoustical stimulation in the normal speech acquisition process of very young children with profound hearing impairment, (d) conduct preliminary studies on brain mechanisms underlying normal speech activity, (e) develop plans for a computing system for speech research, and (f) prepare a bibliography on speech acquisition.

Contract Title: The psychologist in the school: Exploration of a new role for the study of child development in relation to to the educative process. (PH43-65-996)
Contractor: Bank Street College. Herbert Zimiles
Money Allocated: $28,815 (Continuation of FY 1965 funds)

A model for the functioning of a psychologist in interaction with an educational staff to enhance the growth supporting quality of a school as a learning environment for children is anticipated from this contract. It is expected that substantive knowledge of the learning and teaching process will

also be obtained. The effectiveness of the interaction of staff and the psychologist on the learning processes, assessment of children, curriculum planning and major issues of development as they affect learning and educational decisions are to be determined.

Contract Title: Contract to publish and distribute a supplement to Acta Pediatrica entitled "Upper Respiratory Actions of Infants". (PH43-66-28)
Contractor: Almquist and Wiksells Boktrycheri AB, Uppsala, Sweden
Money Allocated: $3,534

This contract has provided for the publication and distribution of a supplement to Acta Pediatrica entitled "Upper Respiratory Actions of Infants" consisting of the four articles:

Cry Sounds of the Newborn Infant

Cry Motions of the Newborn Infant

Studies of Neo-natal Transition: Correlated Cineradiographic and Visual-Acoustic Observations

Distortions of Upper Respiratory and Swallow Motions in Infants Having Anomalies of the Upper Pharynx

Contract Title: Conference on Perceptual development in relation to theories of intelligence and cognition. (PH43-65-1010)
Contractor: Institute for Juvenile Research, Department of Mental Health, Chicago, Illinois. Dr. Noel Jenkin and Robert H. Pollack
Money Allocated: $10,400 (Continuation of FY 1965 funds)

This conference brought together scientists from several fields to formulate a useful body of normative data to give a picture of perceptual development from birth to maturity.

Contract Title: Conference on research and teaching of infant development. (PH43-66-89)
Contractor: Merrill-Palmer Institute, Detroit, Michigan. Irving E. Sigel
Money Allocated: $3,380 (Continuation of FY 1965 funds)

The purpose of this contract was to conduct a conference on research and teaching of infant development. The conference provided a medium of interaction between researchers in infant development and educators who teach human development as well as between researchers.

Contract Title: Provide indexing of speech and hearing literature from January 1, 1965 (PH43-65-21)
Contractor: Gallaudet College
Money Allocated: $7,500 (Continuation of FY 1965 funds)

This contract provided for the indexing of the speech and hearing literature from January 1965 thus providing a completed index from January 1, 1960 onward. The indexed material is being transferred to IBM cards as was done with that information obtained with Contract No. PH43-65-637.

New contracts under review for FY 1966 funding:

(1) A contract with the Children's Hospital Research Foundation, Washington, D. C. to study the role of viral infection in precipitating "Sudden Death" in infants. The explanation for the sudden death syndrome remains in the realm of speculation. The last studies of significance were conducted prior to the recovery and identification of many of the respiratory viruses known today. It is proposed that the early identification of cases of sudden death will be made known to the contract team so microbiological studies for viral and bacterial recoveries including the mycoplasma may be initiated on the deceased infant and members of the family. Fluorescent antibody studies will be conducted on target tissues. Tissues will be stored for subsequent studies. Epidemiological studies will be included. Consideration will be given to determining the cause of death over and above the host-virus relationship.

(2) Continuing the critical evaluation of longitudinal studies by this Institute, the Department of Pediatrics at Temple University will sponsor a three-day study session bringing together scientists from various biological and behavioral disciplines who have in common the need to analyze and evaluate the "normal" changes in human populations over time.

(3) A contract with Michigan State University, Department of Psychology, will provide a developmental psychologist to work with the Harvard-Florence study for about six months to determine the completeness of the behavioral science data on the subjects and assess the value of this data for longitudinal studies. An effort will also be made to determine the possibilities of integrating the behavioral measures with systematic information on physical growth and development. The Harvard-Florence study will be a completely unique project where longitudinal-cross cultural research may be conducted. This contract will aid in evaluating the environment of this research potential.

NICHD annual Report
July 1, 1965 through June 30, 1966
Growth and Development Program

Biological Sciences Branch

The intramural Biological Sciences Branch has five major sections under consideration: (a) developmental biochemistry including biochemistry of differentiation and neurochemistry, (b) developmental immunology, (c) developmental physiology including neurophysiology, (d) nutrition, and (e) physical growth.

Michael Frank, M.D. is spending this year working in developmental immunology with Dr. J. Humphrey at Mill Hill, London, England. He will be returning to the Institute within the next few months to continue the research which he initiated here several years ago and which he extended while he has been in London.

W. H. Glinsmann, M.D., working in the nutrition section of the Biological Branch, is being accommodated by the laboratories of NIAMD and is collaborating with G. E. Mortimore, M.D. for part of his research activity. Both Dr. Glinsmann's own research and the collaborative research report follows. The collaborative research will also appear in Dr. Mortimore's report from the Section on Diabetes and Intermediary Metabolism, NIAMD.

Behavioral Sciences Branch

The intramural Behavioral Science Branch has five major sections under consideration: (a) physiological correlates of behavior, (b) cognition and learning, (c) social development, (d) personality development, and (e) psycholinguistics.

Research in only the personality development section has been initiated.

Additional intramural staff are being actively recruited.

Serial No. HD-GD1
1. Nutrition Section
2. Biological Branch
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Regulation of Carbohydrate Metabolism

Previous Serial Number:

Principal Investigator: W. H. Glinsmann, M.D.

Other Investigators: W. Mertz, M.D., F. Feldman, Ph.D., and J. L. E. Ericsson, M.D.

Cooperating Units: Walter Reed Army Institute of Research, and Karolinska Institute, Stockholm

Man Years (computed for the 12 month period)

Total: One
Professional: One
Others: None

Project Description:

I. The metabolism of trivalent chromium (Cr III). In vitro and in vivo studies in the rat have indicated that Cr III is an essential glucose tolerance factor which may act by facilitating insulin binding to insulin-sensitive membranes. Previous studies with trace supplementation in normal and diabetic subjects would support that it may also be important for optimal glucose utilization in man. On the assumption that chromium normally is involved with insulin, mobilization and transport via a plasma intermediate may be anticipated. Initial studies have been completed which show that plasma chromium does rise with glucose loading in normal subjects but may not when an impairment in glucose tolerance is present. Other investigators are now pursuing the relationship between alterations in plasma chromium, glucose utilization and plasma insulin and insulin-like activity. Since tissue chromium levels in the U.S. are known to diminish significantly with increasing age, studies on the effect of Cr III on the impaired glucose tolerance that occurs with aging are planned to determine the relationship of this impairment to a possible Cr III deficiency.

II. Studies on the regulation of hepatic protein metabolism, and

III. Regulation of carbohydrate metabolism in the isolated rat liver are reported in the progress report from CEB, NIAMD - Mortimore and Glinsmann. (Copy of report follows.)

IV. Alterations in subcellular structure of hepatic parenchymal cells associated with hormonal responses in the isolated rat liver. In recent studies in the rat the relationship of subcellular organelles and the changes following exposure to hypoxic environments were studied. Particular attention was focused on the development and fate of the primary (Golgi derived) and secondary ("autophagic vacuoles" or cytosegresome") forms. Their formation and turnover was shown to be rapid and not associated with cell death and a role in normal catabolic processes was suggested. Under current investigation are the alterations in hepatic fine structure following insulin and glucogon administration in a hormonally responsive isolated perfused liver preparation. (See parts II and III.) Particular attention will be directed to the mitochondrial and lysosomal changes associated with glycogenolysis and alterations in protein breakdown. Control observations will also provide important information about the viability of the basic preparation.

Publications:

1. Glinsmann, W. H. and W. Mertz: Effect of Trivalent Chromium on Glucose Tolerance. Metabolism, in press, June, 1966.

2. Glinsmann, W. H., F. Feldman, and W. Mertz: Plasma Chromium During Glucose Loading. Science, in press, 1966.

3. Mortimore, G. E., E. King, Jr., C. E. Mondon, and W. H. Glinsmann: Effects of Insulin on Net Carbohydrate Alterations in the Perfused Rat Liver. Am. J. Physiol., in press, 1966.

4. Ericsson, J. L. E. and W. H. Glinsmann: Observations on the Subcellular Organization of Hepatic Parenchymal Cells. I. Golgi Apparatus, Cytosomes and Cytosegresomes in Normal Cells. Laboratory Investigation 15: 1966.

5. Glinsmann, W. H. and J. L. E. Ericsson: Observations on the Subcellular Organization of Hepatic Parenchymal Cells. II. Evolution of Reversible Alterations Induced by Hypoxia. Laboratory Investigation 15: 1966.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on the Mechanism of action of insulin and glucagon in the liver

Previous Serial Number:

Principal Investigators: G. E. Mortimore, M.D. (NIAMD) and W. H. Glinsmann, M.D. (NICHD)

Other Investigators: Elmore King, Jr.

Cooperating Units: None

Man Years (computed for the 12 month period)

Total: Two
Professional: One
Other: One

Project Description:

I. Studies on the regulation of hepatic protein metabolism. In an earlier report insulin was noted to inhibit net protein breakdown by about 50 per cent in the isolated, perfused rat liver. The results of subsequent experiments with livers prelabeled with L-valine-1-C^{14} in vivo suggested that much of the net effect might be explained by an inhibition of protein degradation rather than a stimulation of protein synthesis. A model was devised by which it was possible to estimate alterations in protein degradation from the rate of release of valine (R) over short periods of time. R was calculated from the following

$$R = \frac{-(S-S_o)}{t} \left[\frac{\log a/a_o}{\log S_o/S}\right]$$

where a is the specific activity of perfusate valine and S is a direct estimate of the rapidly mixing valine pool, which comprised more than 90 per cent of total free valine. Since valine is neither synthesized nor metabolized in liver to any appreciable extent, the apparent incorporation of valine into protein may be calculated from the difference between R and the net change in valine. The continuous infusion of insulin at 2.4 ug/hr inhibited R by about 40 per cent. Glucagon at 10 ug/hr increased R slightly when given alone, but in the presence of insulin protein degradation was enhanced by nearly 60 per cent. Neither hormone affected the rate of entry of perfusate valine into

liver of serum protein. Protein breakdown was not influenced by glucose concentration between 1.0 and 4.0 mg/ml, but it was reduced when red cells were omitted from the medium.

Since the quantity of protein represented by these hormonal changes is relatively large, these effects may be assumed to have considerable importance in the regulation of protein in vivo. Studies in progress indicate that the inhibitory effect of insulin is not limited to liver. Amino acid release by the perfused rat hind-limb was found to be suppressed strongly by insulin under conditions in which no stimulus to valine incorporation into protein could be seen. While the manner by which insulin and glucagon exert their influence is not presently known, the possible relationship between protein degradation and alterations in cyclic AMP and K^+ transfer is presently under study.

II. Regulation of carbohydrate metabolism in the isolate rat liver. Hepatic glycogen is known to break down rapidly in vitro. Although it may be stabilized to some extent by high concentrations of glucose, it is possible that a major factor in its instability is the limitation in the supply of oxygen to the surviving tissue. The present study was undertaken to test the effect of varying concentrations of washed sheep red cells on net carbohydrate alterations in the isolated, perfused rat liver. When livers were perfused with oxygenated Krebs-Ringer bicarbonate buffer alone, glycogen depletion was rapid and the loss was nearly complete by 60 min. The addition of red cells decreased the breakdown markedly and the effect was roughly proportional to the increase in oxygen uptake. The largest change occurred with the first addition of red cells and the effect leveled off at normal packed cell volumes. When livers were perfused without recycling with a medium free of glucose, the release of glucose and lactate by the liver was not affected by red cells or increased after gassing the medium with nitrogen. These findings suggest that (a) hepatic glycogenolysis is not influenced by the availability of oxygen as is the case with muscle and (b) that the red cell effect was localized at an early step in glucose utilization which would be at sites regulating glucose entry into the cell and/or its subsequent phosphorylation. This is further supported by the finding that the red cell effect was clearly glucose concentration dependent. The fact that the carbohydrate balance across the liver was not influenced by high concentrations of 3-0-methyl glucose substantiates earlier views that a competitive sugar transport, as described in other tissues, is not present in liver. Thus phosphorylation is probably the rate-limiting step most strongly affected by oxygen lack. Inasmuch as the inhibition by insulin of net glucose release by the perfused liver is not glucose concentration dependent, this hormonal regulation may well be localized at sites within the glycogen cycle rather than at steps regulating glucose phosphorylation.

Publications:

1. Mortimore, G. E., E. King, Jr., C. E. Mondon, and W. H. Glinsmann: Effects of Insulin on Net Carbohydrate Alterations in the Perfused Rat Liver. Am. J. Physiol. (In Press) 1966.

2. Mondon, E. E. and G. E. Mortimore: Effects of Insulin on Amino Acid Release and Urea Formation in the Perfused Rat Liver. Am. J. Physiol. (In Press) 1966.

Serial No. HD-GD3

1. Personality Development Section
2. Behavioral Branch
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies of intellectual and personality development of children from culturally deprived backgrounds

Previous Serial Number:

Principal Investigator: Leon Yarrow, Ph.D.

Other Investigators: Michael Fishman, M.D.

Cooperating Units: Project Head Start

Man Years (computed for the 12 month period)

Total: One
Professional: One
Other: None

Project Description:

During fiscal year 1966 a personality development section has been set up within the Behavioral Sciences Branch of the Growth and Development Program. Research in this section will encompass descriptive and analytic studies of a broad range of problems including the following: development of interpersonal competence; development of feelings of adequacy; learning appropriate sex-role behavior; acquisition of values and incorporation of ethical standards; internalization of behavior controls; development of characteristic styles of coping with normal and stressful environmental demands.

Current research activity of the section is concerned with four related problems: analysis of the components of learning environments of preschool children; delineation of the characteristics of children from deprived environments; studies of relationships between specific environmental variables and changes in intellectual and personality characteristics; and studies of the interrelationships between cognitive and personality functions of preschool children. The initial work in these areas involves relatively small groups of children with the focus on methodology. It is being carried on with subjects from Head Start nursery school programs and forms part of a larger program on personality development, which will involve children of varying social backgrounds.

It was recognized at the time that the personality development section first took form that disadvantaged children can highlight variables important

for understanding environmental influences on normal development. Therefore, the Project Head Start program presented a particularly useful potential resource for the section. With this in mind, the Growth and Development Program Director and the section staff met with Dr. Julius Richmond, the Program Director for Project Head Start, and Dr. Edmund Gordon, Head Start's Research Director, during the summer of 1965 to discuss the possibility of research activity with Head Start populations. The meeting produced an enthusiastic endorsement of such efforts. Following the meeting, preliminary evaluative visits were made to Head Start summer programs by the section staff and a psychologist, contracted by the staff, to assess ways that particular research objectives might be pursued in such settings. The next steps involved a series of contacts with local officials which resulted in local clearance for the work to proceed. The final step was direct contact with each of two Head Start programs in the District of Columbia with which working arrangements now exist.

While working agreements were being completed, the section staff proceeded to develop plans for the pilot program currently under way. Consultative meetings with Dr. Lois Murphy and Dr. Irving Sigel, and attendance at a national meeting of those doing research with Head Start populations aided the staff in formulating the approach presently being employed.

The staff is being aided in its ongoing work by a group of four research assistants who are participating in the collection and analysis of data. All four have graduate training and experience in psychology and/or child development.

Data are being obtained at present from two major sources; observation of playroom activities of the children, and a battery of established testing procedures administered in several sessions with the subjects. A rating scale, constructed specifically for use in the work under way, is being employed to assess behavior during playroom sessions. A number of different procedures are being used in the test battery including the following: Stanford Binet, Peabody Picture Vocabulary, Goodenough, Seguin board, CAT, Maccoby Motor Inhibition, Sigel Sorting. In addition, systematic observation will be made of group interaction in Head Start classrooms.

The work outlined above will serve to further develop methodology and refine concepts which will be utilized in larger scale investigations relating to the interaction of environment and personality development and the interrelationship of cognition and personality.

NICHD Annual Report
July 1, 1965 through June 30, 1966
Aging Program
Program Summary

The Aging Program supports research and training for research that bears on the aging process and the developmental and retrogressive changes that occur during the adult years. It is thus concerned with problems of the entire adult lifespan as well as problems of the elderly. The program supports a wide range of disciplines that are concerned with various aspects of aging. These disciplines can be divided into four major areas of interest; Molecular and Cellular Biology, Comparative and Human Biology, Behavioral Sciences, and Social Gerontology.

As of March 31, 1966 there were 63 research grants, 9 training grants, 7 fellowships, 4 career research development awards, and 1 career research award being supported by the Aging Program. In fiscal year 1966 the expenditure* for research grants was $3,478,000 and that for training grants was $455,000. The total expenditure on all types of grants was $4,311,000. Funds for direct research were $1,268,000 and those for contract studies were $370,000. The total expenditure by the Aging Program was $6,089,000.

The research grant structure of the Aging Program is unusual in that 7 of the 63 research grants are program project grants. These 7 grants account for about half of the funds spent on research grants. They are large, complex programs that provide visibility and focus for studies of aging in favorable research environments. They are located at Duke University, the University of Miami, Brown University, Western Reserve University, Yeshiva University, New York University, and the Lovelace Foundation for Medical Research and Education.

During the fiscal year 1966 the Gerontology Branch in Baltimore, Maryland was transferred from the National Heart Institute to the National Institute of Child Health and Human Development. This activity is currently being carried on in laboratories and wards in the Baltimore City Hospitals. A building to house this activity is currently being constructed and should be ready for occupancy about October 1967.

One vital area of the research carried out under the auspices of the Aging Program deals with the basic biology of aging. In the past two decades there have been developments that augur well for an increased understanding of the basic biology of aging. There has been a transformation of our knowledge of the extremely minute structure of the cell brought about by electron microscopy and its ancillary techniques. Our knowledge of the genetic mechanisms through which the daily activities of cells are controlled has increased greatly. The entire body of information that deals with chemical changes in cells and their bases in cellular structure has expanded greatly. It may be that an understanding of the basic biology of aging may be found in this area involving cellular biology and now under investigation by morphologists, geneticists,

*All estimates of expenditures were made in April 1966.

biochemists, and biophysicists. It is extremely important to an understanding of the basic biology of aging that work in these fundamental areas continue and that the concepts and techniques of these fields be applied to the study of the aging process.

One possible mechanism of aging deals with changes in macromolecular structures that are replaced very slowly. One such protein is collagen. Evidence has accumulated that chemical cross-links in collagen increase with increasing age. This may have some importance from the standpoint of the function of collagen. In addition similar changes may take place in proteins less accessible to study than collagen. The Aging Program is supporting the work of several investigators interested in collagen and in changes in collagen with age. One project has shown that an aldehyde forms one of the cross-links that occur in collagen. Work is being carried on with several agents that reduce cross-linking to see if such a reduction has any effect on other manifestations of aging.

It is possible that during aging errors develop in the genetic deoxyribonucleic acid (DNA) which contains the information that directs the metabolic activities of the cell. One current theory is that this is the fundamental mechanism of aging. If errors do occur in DNA with increasing age, then one might expect these errors to lead to the formation of defective ribonucleic acid (RNA) which in turn would lead to defective enzyme function. These defective enzymes might not suppress the rate of formation of RNA as normal ones do. Thus there might be a greatly accelerated production of RNA. This possibility is being investigated in several laboratories.

As cells grow old they may fail to excrete all the metabolic products that they produce. One line of evidence suggesting that this is the case is the accumulation of insoluble, brown material in some of the cells of older animals. Work by several investigators on these pigments is being supported. The pigments are being studied from the standpoint of their chemical composition and their mode of production. One theory that is emerging as a result of these studies is that the cellular lysosomes release enzymes that digest cellular constituents and leave an indigestible residue of lipids and other products which form the pigment.

Still another approach to the basic biology of aging suggests that with increasing age there is an increased production of antibodies against the bodies own cells. It is known that the autoantibodies in the plasma increase with age and that the incidence of amyloidosis increases with age. The Aging Program is supporting research bearing on this approach.

A study on the rates of oxidation by young and senescent rats of various compounds labeled with radioactive carbon has been supported. The overall results and conclusions from the study may be summarized as follows: Acetate-2-^{14}C is oxidized more rapidly in senescent rats than in adult rats. No significant age-associated differences in oxidative rates were observed with several other compounds including acetate-1-^{14}C, octanote-1-^{14}C, propionate-2-^{14}C, D-glucose-1-^{14}C, succinate-2-^{14}C, DL-alanine-3-^{14}C, and DL-glutamate-3-4-^{14}C. The results have suggested that there may be an alteration with advancing age in the metabolic control mechanisms involving

mitochondrial respiration and the extra-mitochondrial biosynthetic mechanisms. Studies on the regulatory mechanisms, possibly involving changes in concentrations of intermediates, allosteric alterations of enzyme activity, and hormonal effects will be considered in future research.

One of the recurrent theories in aging research deals with the alterations in tissue permeability with age. There has been much speculation but few direct data on this topic. Recent work under the auspices of the Aging Program have shown that aortic permeability increases greatly with age up to the age of about 45 years. Further studies on the permeability of connective tissue as a function of age will be carried out.

The survival of labeled erythrocytes from young and old rats has been studied in young and old rats in work supported by the Aging Program. It has been found that neither the age of the donor or the age of the recipient has any effect on the survival of the erythrocytes.

There are many biologic problems that arise as a result of the aging process and that manifest themselves at the level of the organ systems that comprise the body and at the level of the intact organism. These problems in the human can be effectively investigated by cross-sectional and longitudinal studies. The Aging Program is supporting several such studies. One of these is the study being conducted by the Gerontology Branch in Baltimore. About 600 participants have been involved in this study. They have been examined from 1 to 6 times. Measurements of biochemical, physiological, and psychological variables have been made. One of the very interesting findings of this study is that carbohydrate tolerance decreases progressively with age and that beyond the age of 45 years half of the subjects studied have glucose tolerance curves that by the standards applied to young adults would be considered diabetic. In the past year these subjects have been shown to have a smaller decrease in plasma glucose following intravenous tolbutamide than young subjects. This represents further evidence of impairment of carbohydrate metabolism with age.

Another longitudinal study is being carried out at the Lovelace Foundation for Medical Education and Research in Albuquerque, New Mexico. A series of physiological and psychological tests are performed at intervals in a population of about 500 professional airplane pilots. These tests include measurements of cardiovascular function, renal function, pulmonary function, and various types of psychological function. Preliminary analysis indicates not only that there is progressive loss of function with age but that the degree of physiological loss correlates with the degree of psychological loss.

In addition to the above and other studies which include cross-sectional and longitudinal components, some purely cross-sectional studies are being carried out in men and experimental animals. Purely cross-sectional studies have the advantage of permitting a relatively rapid accumulation of information though they will not answer certain types of questions.

One of these studies has shown that older subjects are unable to correct an induced acidosis as rapidly as young subjects. This is due to an impairment of excretion of titratable acidity and ammonia in the urine.

Studies on the ability of the isolated rat heart to perform work have unequivocally confirmed the previous findings by the same investigators of a significant decrease in cardiac capability with age. Studies to determine the mechanism of this decrease in capability are continuing.

The Aging Program sponsors a number of analyses of age-related differences in behavior and of relationships between age and psychological, sociological, physiological, and medical variables. Work is being carried out both on humans and on rodents. Work sponsored by the Aging Program and elsewhere shows a welcome trend away from a mere cataloging of age-related differences in behavior and toward analyses of the nature of these differences and the development of techniques to optimize psychological capacities in the older organism.

An abundant literature indicates that older humans usually learn verbal associations much less rapidly than young adult humans. Recent experiments supported by the Aging Program indicate that much of this apparent deficit is due to a reluctance to respond which in turn appears to be related to physiological over-arousal or "anxiety" as defined by autonomic measures. Present work on these variables indicates that verbal-associative learning in older humans can be improved greatly by allowing more time in which to respond, perhaps by blocking autonomic activity by drugs, and perhaps by manipulating the older learner's expectancies of the time available for responding.

Older people also have been reported to perform more poorly than young on certain kinds of logical problems which require logical inferences and the discovery of concepts or rules and require a considerable short-term memory load. Studies on the qualitative work patterns and "strategies" of young and old humans are being supported.

Several investigations are dealing with the "information processing" rates and techniques of younger and older humans in a variety of laboratory tasks. Although older people are usually found to be slower, and sometimes poorer than the young in most such laboratory tasks, one of the Aging Program's grantees is testing the hypothesis that the human's ability to learn the patterns in a flow of information increases with age throughout most of the working life. Other investigators are dealing with a variety of other relevant variables, such as short-term memory load.

Several projects concerned with psychological task performances and concurrent psychophysiological indices of arousal and stress, such as electroencephalographic patterns, autonomic changes and free fatty acid mobilization are being supported. The effects on behavior of manipulating autonomic activity by chemical means, in young and old humans are also being studied. Other longitudinal studies in humans relate changes in intelligence test scores and personality measures across time with changes in health status, electroencephalogram, chromosomal changes, and other physiological and biological measures.

It has been demonstrated that a sudden change in environment--such as being moved from one institution to another--produces a substantial increase in death or the development of depression and other psychiatric conditions in elderly humans. A large group of elderly people scheduled to enter institutions will be studied from one year before to one year after institutionalization, with

emphasis on personality structure and psychiatric status, in an effort to mitigate this unfortunate effect. A scale of "attitudes towards the elderly" is being developed in order to counsel those on whom the elderly person is dependent.

A less well-developed area for research carried out under the auspices of the Aging Program is that of social gerontology. Investigations in this area focus upon developmental or retrogressive reactions of whole persons rather than upon alterations in abstracted processes such as verbal association, visual acuity, or a particular motor response. Investigators tend to view persons within their existing environmental contexts. Of particular relevance are the effects of economic, physical, physiological and social background factors, and changes in them which are usually concomitant with advancing chronological age in present-day society. Such investigations will help to clarify knowledge of aging through differentiating those changes determined by chronological age from those consequent upon alterations in environmental influences, which are commonly concurrent with advancing age in our culture.

One NICHD-supported investigator analyzed some effects of the well-documented low income level of older people. He studied the ways in which older persons used their more limited incomes, whether the amounts they spent for various categories of consumptions were adequate for their needs, and ways in which the patterns of expenditure of the aged differed from those of younger segments of the population.

Though aging and illness are not synonymous, the incidence of chronic ailments is much higher for people over 65 than for those younger. An investigator found that, while older people were generally aware of the increased likelihood of their incurring such illness, only a small percent expressed concern. Concern about illness was not significantly related to objective or subjective health evaluations, but probably was influenced by the security of these old persons in family life, religion, a stable environment, and financial resources.

Results of another study are consistent in showing that socioeconomic variables are related to reported health status. Tendency to report chronic health conditions was related to labor force status, financial position, and education. More specifically, blue collar workers tended to report illness more frequently than white collar workers, the higher education groups reported less illness than persons at lower educational levels, and higher income groups report less than the lower income groups.

Great emphasis has been placed on the training area in the last year. The amount of money approved for training in aging by the National Advisory Council has tripled in the last year. The total number of training grants approved has increased from 5 to 15.

Plans are being made for a conference of the directors of the training grants and a preliminary meeting to plan for this was recently held.

Several conferences have or will be supported in fiscal year 1966. A three-day symposium on aging was supported at the Salk Institute in November

1965. A number of distinguished biologists interested in the basic biology of the aging process presented their work and participated in a general discussion of the problem. A seminar on genetic mechanisms in aging was held at the annual meeting of the Gerontological Society in November 1965. Interested scientists from this country and abroad discussed this topic which is at the heart of several current theories of aging. The grant mechanism is being used to support the travel of 100 scientists interested in the aging process and the elderly to the International Congress on Gerontology to be held in Vienna in late June 1966. These scientists will participate in and make possible a number of pre-congress colloquia to be held near Vienna. These conferences will cover topics in Biological Sciences, Clinical Medicine, Psychological and Social Sciences, and Applied Social research.

The Aging Program has collaborated with the Information Center of the Communications Branch to help in developing a system that will permit the storage and retrieval by abstract journals and computerized techniques of information bearing on aging. All areas from cellular biology to social gerontology will be covered. It is believed that the ability to retrieve information rapidly and comprehensively will be a major aid to scientists in the field of aging.

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July 1, 1965 through June 30, 1966
Aging Program
Contract and Collaborative Research Summary

The contract mechanism has been used to deal with the problem of maintaining continuity in the bibliographic record on aging research until the Aging Information Center becomes fully operative. One contract with the Gerontological Society and one with Excerpta Medica have permitted this continuity to be maintained. The Aging Information Center will eventually replace them. The Center will scan, record, and abstract biological and behavioral literature on aging. The references and abstracts will be published in a suitable journal and will also be recorded on tape for search by computer for specific types of information.

The contract mechanism has been used to support conferences that are of especial programatic interest but which would probably not be undertaken through the grant mechanism. Two such conferences are the one held at the Salk Institute and the one to be held at the Mayo Foundation. Such conferences will be considered in the future as the need for them arises.

The contract mechanism has been used to obtain administratively useful information. The contract for the preparation of a directory of resources in gerontology, and the contract for the development of information on training needs were for this purpose. Need for the contract mechanism for assembling this type of information will continue although the specific items studied will vary from year to year.

The contract mechanism has been used to support a study to determine whether NICHD should support the development of a handbook covering all phases of human development. Additional work may be considered in connection with the proposed handbook if the preliminary study indicates that such work is worthwhile.

Contract Title: Publication of "Current Publications in Gerontology and Geriatrics"

Contractor: Gerontological Society, Inc.

Money Allocated: $13,274

The objective of this contract is to assemble a bibliography of research in the field of aging consisting of approximately 3,000 citations and to publish this quarterly "Current Publications in Gerontology and Geriatrics" in the Gerontological Society's publication "Journal of Gerontology." This bibliographic information has been published in the Journal of Gerontology for many years and provides a readily accessible source of information on research in aging. Eventually when the Aging Information Center of the NICHD is in full operation, the bibliographic service provided by this contractor will be discontinued.

Contract Title: Translate and Publish Gerontology and Geriatric Abstracts

Contractor: Excerpta Medica Foundation

Money Allocated: $12,307.50

Under this contract, approximately 3000 biomedical journals, in addition to books, reports and other appropriate publications related to the basic and clinical sciences in gerontology and geriatrics, are reviewed and abstracted for publication in the monthly issues of Gerontology and Geriatrics published by the Excerpta Medica Foundation. Abstracted publications include Soviet and Japanese biomedical journals to which the Excerpta Medica Foundation has specially-arranged access. In addition to the monthly publication a cumulative twelve-month author and subject index is prepared and published.

This abstracting service provides an essential, current informational resource to all those working in the field of aging. Within the next year these services will be taken over and expanded by the new Aging Information Center of the NICHD.

Contract Title: Three Seminars and a Symposium on the Biology of Aging

Contractor: Salk Institute

Money Allocated: $3,800

The objective of this contract which was initiated in fiscal year 1965 and has been supplemented for fiscal year 1966 was to conduct three seminars and a symposium on the basic biology of aging and to prepare the manuscripts and discussion of the symposium for publication.

The seminars and symposium were held at the Salk Institute in La Jolla, California. Each of the seminars consisted of a presentation by a distinguished scientist of work in his field and of discussion by a number of scientists invited by the Aging Program of the NICHD and by the fellows of the Salk Institute. The final symposium lasted for three days and consisted of presentations by an outstanding group of scientists that included two Nobel Laureates. The scientists included both biologists with a major interest in aging and biologists whose work was highly relevant to the subject of aging. The presentations defined the status of our present knowledge of many aspects of the biology of aging and suggested new avenues of approach. The manuscripts and a transcript of the discussion have been edited by one of the participants. Arrangements are being made for publication of this material.

Contract Title:

Contractor: Mayo Foundation

Amount Allocated: $5,050

A two-day conference entitled, "A Study of Psychological Reactions to Physiological Changes in Aging," will be directed by Dr. Wendell Swenson of the Mayo Foundation.

Experts in the effects of human aging from both medical and behavioral discipline will be brought together to discuss the title-topic and to exchange information, plans and hypothesis in this area, with the objective stimulating research and achieving a better understanding of the phenomena of aging. An equally important objective is to recruit superior young investigators into research on aging.

The proceedings of the conference will appear in the Proceedings of the Mayo Foundation.

The Conference will be held in the Autumn of 1966.

Contract Title: Directory of International Resources in Gerontology

Contractor: Gerontology Society

Money Allocated: $35,000

Under this contract, the Gerontological Society is undertaking to develop a comprehensive directory of international scientific resources in gerontology. The directory will consist of listings and descriptions of:

a. Personnel (including appropriate biographical data) classified according to research and training interest;

b. Personnel classified by alphabet and by geography in a locator index;

c. Organized research centers;

d. Organized training centers;

e. Scientific and professional organizations with special committees and functions devoted to research and/or training in aging;

f. Specialized and unusual resources such as animal colonies, special equipment, collections of unusual materials.

The information to be included in this directory is now being collected and includes biographical and other informational material from recognized gerontologists and associations concerned with gerontology in the United States and throughout the world. As the data is received by the Society, it is evaluated, collated and cross-indexed with the assistance of committees of experts. Within the next year the data will be prepared for publication. To carry out this extensive effort the Society is working closely with the International Association of Gerontology as well as with other specialized societies and organizations around the world in identifying individuals and facilities relevant to research in aging.

The Aging Program is particularly interested in the development of this directory for it will be an invaluable resource in furthering the development of research in aging. It will facilitate (1) the coordination of gerontological research by putting investigators with similar interests in contact with each other, (2) the stimulation of cross-national research, (3) the broadening of the base of information about training opportunities, and (4) the rapid identification of universities, institutions and individuals who could participate in enlarged programs of training and research in gerontology. The publication will

be widely publicized and distributed throughout the world to interested investigators, associations, and institutions. It is anticipated that the directory will be revised frequently since gerontology is a rapidly changing field.

Contract Title: A Study of Susceptibility to Health Fads and Fallacies

Contractor: NICHD has a reimbursable agreement with the Food and Drug Administration to partially support this contract.

Money Allocated: $25,000

A contract will be let by the FDA to study this matter by interviewing samples of individuals who have complained of being victims of health frauds, and other apparent victims identified by county medical organizations and other groups. The study will emphasize the elucidation of dangers of health fraud to the elderly. Preliminary forms of interview schedules ("questionnaires") have been prepared by the staff of Dr. Moehlmann of the Division of Consumer Education.

This project will be of particular help in promoting the health and welfare of the aged, who tend to be susceptible to health misrepresentations.

The Contract will be awarded prior to June 30, 1966. The contractor's completed report should be delivered by September 30, 1967.

Contract Title: Production of a Report on Gerontological Training Needs and Mechanisms

Contractor: Gerontological Society

Money Allocated: $41,500

Project Description:

Objectives: The purpose of this contract is to conduct a survey and produce a report on the research training needs and mechanisms in gerontology. The report will contain the following:

a. A digest of expert opinion at the specialist level in the relevant sciences.

b. A description of the typical current training program with an analysis of their strengths and weaknesses leading to suggestions for further improvements in research training programs.

c. A projection of the number and type of trainees required in each specialty area and a determination of the characteristics required of these trainees.

The report will be divided into four major sections:

a. Molecular and Cellular Biology
b. Human and Comparative Biology
c. Behavioral Sciences
d. Social Gerontology

Each section will contain reports from the separate disciplines comprising that section as well as an overview or synthesis of the issues for all of those disciplines.

Methods Employed: Conferences of distinguished scientists and educators interested in aging, survey by mail, questionnaires of institutions involved in training, and intensive interview of personnel at selected training sites.

Major Findings: None during the calendar year.

Significance to Biomedical Research and the Program of the Institute:
The present status of research training in gerontology, as well as future needs, have been clearly identified as of major concern to this program.

Proposed Course of Project:

1. Chairmen and committee men for the various sections were secured from among scientists and educators distinguished in their particular disciplines. In various formal and informal conferences these committees prepared a questionnaire.

2. This questionnaire was mailed to members of the Gerontological Society.

3. On the basis of results an intensive interview schedule has been prepared.

4. Experienced interviewers will visit training sites, which are being selected also on the basis of the mailed questionnaire results.

5. A summary report will present findings by sections and if appropriate in an overview with such recommendations as are generic to training in the field of gerontology as a whole.

Contract Title: Preparation of a Report on the Content of a Proposed Handbook on Human Development

Contractor: University of Washington

Money Allocated: $24,000

Project Description:

Objective: A report which would contain an outline of procedures and the details of plans for preparing a comprehensive handbook of human development as an activity of the National Institute of Child Health and Human Development. The report will estimate the feasibility and desirability of such a volume, describe the range of content, name potential authors, outline editorial procedures for content and style, delineate responsibility of functions of scientific and professional advisory committees, describe the composition, functions and reporting responsibility for all committees and individuals having a role in the project.

Methods Employed: Bibliographic and consultative

Major Findings: None during the calendar year

Significance to Biomedical Research and the Program of the Institute:
The handbook under consideration would be valuable to:

1. To organize scattered literature.

2. Juxtapose material from many scientific disciplines related to the same problems.

3. Provide a logical framework for a large portion of current research pertinent to the goals of the NICHD.

4. Give a visible scientific context for current research on development in aging.

5. Encourage teaching and stimulate new research on human development through scholarly and scientific organization of the information.

This contract is for the purposes of exploring the feasibility and desirability of producing such a volume or volumes and of advising upon the content, editorial procedures, potential authors, scientific and professional advisory committees which might be involved.

Proposed Course of Project: The contractor has requested and received an extension, with no additional funds, through June 30, 1966. It is anticipated that the report will be forthcoming by that time.

NICHD Annual Report
July 1, 1965 - June 30, 1966
Aging Program
Intramural Research at Bethesda

Two research projects are being conducted by the staff of the Aging Program at Bethesda. One of these is concerned with the changes in connective tissue with age. Studies carried out under this project have shown that there is a striking increase in the permeability of the aortic wall to albumin with increasing age. Studies directed towards detecting changes in the permeability of other connective tissues are being started. Such changes in permeability of various connective tissues might have considerable significance with regard to changes in the functioning of those tissues with age.

The second research project is concerned with the reaction of elderly people to their housing conditions. Previous studies by the investigator concerned have shown that housing has a great psychological impact on the elderly. The present studies are designed to confirm and extend the previous studies.

Serial No. HD-AG1
1. Aging Program
2.
3. Navy Medical
Research Institute
Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies of Changes in Intercellular Matrix with Age

Principal Investigator: Leroy E. Duncan, Jr., M.D.

Other Investigators: Katherin Lee, A.B.
Almorris Lynch
Barbara Gadegbeku, B.S.

Cooperating Units: None

Man Years (computed for the 12-month period)
Total: 2.83
Professional: .50
Other: 2.33

Project Description:

Objectives: There are numerous intercellular matrices in the animal body. Outstanding ones are the matrices of the skin, ocular vitreous humor, arteries, cartilage, intervertebral discs, and bone. These matrices differ from each other, but share certain characteristics in common. They are made by connective tissue cells and contain mucopolysaccharides and collagenous fibers. They are not static entities but are in a state of continuous turnover which reflects the activity of the cells that create them. They are very important in the support of tissues. In many cases they do this by immobilizing water. Water being almost incompressible can provide structural support if its free movement through the extracellular space is prevented. It appears probable that the turgor of the skin and the vitreous humor is due to the intercellular matrix that they contain. In addition to retarding the free flow of water, it appears probable that intercellular matrix greatly retards the diffusion of solutes particularly plasma proteins through tissues. In vitro, a concentration of hyaluronic acid no greater than that present in some tissues will reduce the diffusion of albumin to 1/20th of its unrestricted value.

There is evidence that intercellular matrices decrease with age. Connective tissue fibrils from young tissue are coated with amorphous jelly; those from old tissues are free of it. Microscopically with techniques which render the ground substance visible by changing the pH of unfixed tissues, young human

tissues can be shown to contain much and old human tissues to contain little intercellular matrix. The quantity of cartilage lining the joints of old animals is decreased; the ground substance of skin is probably decreased; and, the matrix of the vitreous humor, the intervertebral discs, and the osteoid of bone decrease with age. It appears probable that these changes with age form a background for the development with age of many unfortunate processes: osteoarthritis; osteoporosis; retinal detachment due to lack of support by the vitreous humor; and, possibly, increased susceptibility to bacterial spread in various tissues due to the loss of the restraining effect of the matrix.

In addition, changes in the intercellular matrix of the arterial wall may be important in atherosclerosis. The most generally accepted theory of atherosclerosis is the filtration theory. This theory is that plasma lipoproteins enter the arterial wall where they break down and leave a deposit of lipids which form the atheromatous lesions of atherosclerosis. If this theory is correct, then it seems likely that the state of the arterial wall as it affects the diffusion and filtration of plasma proteins through it is important in the genesis of atherosclerosis. An increase in permeability would permit lipoproteins to enter the arterial wall more rapidly. However, their passage through the arterial wall would still be interrupted by the elastic lamellae, and they would accumulate to form atheromata. Thus, the overall effect of an increase in permeability might be an acceleration of atherogenesis. The data of Kirk are in accord with the foregoing concept. He found that the permeability of human aortic intima and media to glucose and smaller solutes increases with age.

Although the changes in intercellular matrices appear to be important in the aging process and to underlie many of the clinical manifestations of aging, it seems probable that they are the result of some changes with age in the connective tissue cells. Also, that this intercellular change should be considered a more fundamental change than the changes in matrix that result from it.

Methods Employed: Obviously a great many approaches to the foregoing problems requiring different conceptual and technical approaches are possible. The approach that has been used during the past year is to study the diffusion of labeled serum albumin into tissue *in vitro*.

Major Findings: Two types of tissue have been used. One is human aortic tissue obtained at autopsy. The site of the aorta that was chosen for study was that area just below the great vessels. Eight small discs were cut from this area from each aorta. The rate of diffusion of albumin into each of these discs and the cholesterol content of each disc were determined. Ninety-five aortas have been studied ranging from the aortas of newborns to those of patients in the ninth decade of life. A striking increase in the permeability of the aortic wall to albumin has been found. The permeability increases logarithmically up to the age of about 45 and undergoes a sixfold increase during this period. Thereafter the permeability is constant. These results are highly significant statistically ($p < .001$). The cholesterol concentration in the aortic wall also increases with age from the first to the ninth

decade of life. It undergoes a tenfold increase during this period. This increase is highly significant statistically. It is interesting that although both the permeability and the cholesterol content of aortic wall correlate with age, they do not correlate with each other. The increased aortic permeability with age is of interest in connection with the views put forward at the start of this project description.

The diffusion of albumin into the skin of young and old rats has also been studied. No difference was found. However, the old rats were not very old.

Proposed Course of Project: The present plan is to study the skin of rats of various ages to determine at what age the ground substance becomes very sparse. This will be done by homogenizing the skin and examining it by darkfield and phase contrast microscopy. Under these conditions the ground substance is visible and in humans at least decreases greatly with age. Once we know how old rats have to be to be grossly deficient in ground substance, we will undertake, once again, a study of the diffusion of albumin into rat skin as a function of age.

Honors and Awards: The principal investigator was invited to participate in a symposium on connective tissue in Semmering, Austria. This is one of the pre-congress colloquia that will precede the International Congress on Gerontology.

Publications: None

Serial No. HD-AG2
1. Aging Program
2.
3.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Effects of Physical and Social Environment on Old People

Previous Serial Number: None

Principal Investigator: Frances M. Carp, Ph.D.

Other Investigators: None

Cooperating Units: San Antonio, Texas, Housing Authority

Man Years

Total:	17/40
Professional:	1/20
Others:	3/8

Project Description:

Objectives: In general the purpose of this investigation is to validate the findings of an earlier study of elderly applicants for public housing by checking them (1) over a longer period of time on survivors of the original subject group and (2) on a different subject sample, (3) using, in some instances, modified instruments.

Methods Employed: In 1960 preliminary data were collected on various demographic, biographic, attitudinal and other psychological test variables on 352 applicants for a new public housing facility (Victoria Plaza) in San Antonio, Texas, which was designed for older people. In the period between 12 and 18 months after original data collection, follow-up materials were obtained.

Data analyses dealt with (1) changes over time (12 to 15 months) which were different for the subjects who were admitted to Victoria Plaza (through normal housing authority procedures) than for those who were not. This analysis used standard co-variance design which allowed for effect of initial differences between groups. (2) Predictors in the base line data were validated against information collected on the 204 residents after they had been in Victoria Plaza about 18 months. In addition, (3) sociometric data were collected at three-month intervals during the first year and a half of residence in the new building, and patterns of social organization were traced.

To test the validity and generality of the earlier findings, it is necessary to determine the following:

A. Did (1) satisfaction and (2) other attitudinal and behavioral changes which differentiated residents from nonresidents reflect a "honeymoon period" or "Hawthorne effect" or, on the other hand, were they enduring results of changes in the life situation?

The prediction is that changes which were different for residents than for nonresidents from 1960 to 1961 will also be statistically significant and in the same direction from 1960 to 1967. Data are being collected for this purpose on all survivors of the original sample.

B. Were (1) changes attributed to impact of changed circumstances of life, and (2) predictors of adjustment among in-movers specific to the subject sample and/or do they apply to other residential facilities and other elderly applicants. Predictions here are that:

a. Variables will show the same pattern of differential change for another group of aged people, applicants for a new public housing facility for the aged in San Antonio, over the period from applicant status to that of 12 to 15 months after those selected for residency move into their apartments.

b. The predictors of adjustment which were developed on the Victoria Plaza residents will be applicable to this new resident group. (Prediction of adjustment, for each of the four criterion measures separately, will be attempted, using the regression weights which were developed on the Victoria Plaza sample. In addition, optimal regression weights for the items will be developed for each criterion.)

Data collection on the cross-validation sample will begin about January 1, 1967.

Significance to Bio-medical Research and to Programs of the Institute

The Impact of Environmental Setting: In the 1960-1961 data, evidence of the dramatic effect of improved life-setting on this group of older people was overwhelming. It appeared not only in increased satisfaction of residents with their living situation but also in more favorable attitudes about themselves and toward others, in signs of improved physical and mental health, and in more active and sociable patterns of life.

Alterations in outlook and life style among the resident group are more clearly attributable to housing because it was possible to view them in relation to those among applicants who had not made the move. The unsuccessful applicants, initially similar in most ways to the successful ones, exhibited little difference in behavior or attitude from 1960 to 1961.

The consistent pattern of change among Plaza residents contraindicates rigidity as a regular component of the aging personality. The behavior of these people calls into question the difficulty, with increasing age as distinct from decreasing opportunities, of forming new social relationships. The results suggest strongly that old people's dissatisfactions with the circumstances of

their lives are realistic reactions to difficult physical and social situations, and that the expressions of discontent cease when the causes are alleviated. The relative consistency of nonresident records from 1960 to 1961 supports attribution of resident change to housing.

Changes exhibited among residents in their attitudes and ways of living were generally toward activity and informal social contact, not at all toward disengagement. The findings suggest that more attention needs to be paid to the role of the setting in determining the experience and behavior of people within it, even people who are old. It points also to the necessity for more careful scrutiny of traits considered to be age-related. Some environmental conditions may so regularly accompany chronological age that their effects are mistaken for those of aging itself.

The rather comprehensive background information on this group suggests that they are much like most old people in this country today. Probably also the fact that the changes came about in a group of individuals who were simply getting along as well as they could as normal members of a community rather than achieving visibility either through acknowledgement of physical or economic helplessness, or identification with some privileged group, supports the prospect of more widespread improvement of the later years of life through changes in the environmental settings in which they are lived.

To clarify the persistence and generality of these findings, the studies currently under way will test them (1) over a six year period in the original subjects and (2) over a 12-15 month period using a different sample of subjects applying to a different housing facility.

Prediction of Adjustment: To minimize utilization of facilities and to guide individuals toward situations into which they will fit most satisfactorily, it is important to develop predictors of "adjustment" to various types of living situations. This is true while facilities are in short supply; it will be true as long as there is variety of accommodation which allows choice on the part of the applicant.

This group of applicants was fairly homogeneous in many ways, since all had preselected themselves by making application for housing and all had passed the Housing Authority's initial eligibility screening. However, this is the sort of group with which selection systems will have to deal in the future, in many cases.

Also, satisfaction with the Plaza was nearly universal, and other indexes of adjustment showed most of the residents to have been successful. Nevertheless, there was sufficient difference among the people on each of these measures of morale or adjustment to allow development of selection techniques which would be useful in screening future tenants for this or a similar facility. The generality of these predictor systems can be validated, of course, only by their application to new data, as is planned in the present study.

Publications: Materials, procedures, data analyses, and results of the 1960-61 study are presented in a book in press, "A Future for the Aged: The Residents of Victoria Plaza," the University of Texas Press, June, 1966.

ANNUAL REPORT OF THE GERONTOLOGY BRANCH

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

JULY 1, 1965 - JUNE 30, 1966

The goals of the Gerontology Branch are to describe in quantitative terms the changes that take place with aging and to investigate the basic biological mechanisms of these changes with a view to reducing the impairments and disabilities of older people. The research program falls into two major categories: (a) the description of biochemical, physiological, and psychological changes that take place with aging, and (b) investigations of the mechanisms of age changes at the social, psychological, clinical, physiological, biochemical, cellular, and molecular levels.

During the past year, members of the professional staff have contributed considerable time and effort to working out the design of many special features of the Gerontology Research Building to be constructed in Baltimore. Construction of this 6.9 million dollar research building started in September 1965. Completion of the building is scheduled for October 1967.

Highlights of findings from the Gerontology Branch which have extended knowledge of aging include the following.

1. Age differences in interference effects in learning are significantly reduced when anticipation time during the original learning is long.

2. Older subjects show an impaired response with respect to blood sugar levels to the intravenous administration of tolbutamide. These results offer further evidence of impairment of carbohydrate metabolism with advancing age.

3. The impairment of glucose tolerance in the aged cannot be ascribed to an increase in free fatty acids in the plasma.

4. Total caloric, vitamin, and mineral intakes diminish with age even in subjects whose diets are not limited by economic factors. In contrast, the intake of vitamin C increased after age 70. In about half of the middle aged and elderly subjects the dietary intake of Ca was less than the NRC recommended intake.

5. A mathematical method has been devised which may make it possible to assess individual differences in non-uniformity of lung ventilation.

6. The impairment in the ability of the aged kidney to form ammonia is not due to inadequate amounts of substrate (glutamine) for its production.

7. A double isotope derivative assay for angiotonin has been developed which permits the quantitative estimation of nanogram amounts of the hormone.

8. A method has been developed which permits the achievement of a steady state of blood glucose levels at any concentration desired by the investigator. This method will be of great value in investigating many problems of glucose metabolism in humans, such as the mechanisms of insulin synthesis and release in response to hyperglycemia.

9. In rats the ability to make fine discriminations in taste diminishes significantly with age.

10. Although the experimental introduction of "errors" by feeding ethionine to rats increases protein and RNA metabolism, no age differences in the response were observed.

11. In rats, enzymes involved in neural transmission and conduction failed to show significant differences between young and old rats.

12. The reduction of food intake in maternal rats has been shown to produce young which grow at a slower rate than normal and fail to attain normal adult size even when fed ad libitum after weaning. This method will be of great value in projected studies of the life lengthening effects of starvation in rats.

13. Under appropriate conditions the degradation of RNA by metal ions such as zinc can be made specific and this specificity can be changed by inhibition of the degradation by another metal ion. Thus non-enzymatic reactions may be utilized for base-sequence determinations of nucleic acids.

14. An additional coupling factor associated with oxidative phosphorylation has been isolated from cardiac mitochondria.

15. Age changes in collagen may be due to a reorientation of existing intra-molecular crosslinks rather than the addition of new crosslinks.

The longitudinal multi-disciplinary study of subjects aged 17 to 103 years has been successfully continued in the past year. The total subject group now numbers 587 self-recruited community-dwelling men. It is 8 years since the study began and we are thus just beginning to examine the earliest volunteers for the sixth time. Half of the group has now had at least 3 visits (at approximately 18-month intervals) and one fourth of the group has had 4 visits. Considering the life span of man and the anticipated rates of change in the various physiological and psychological functions being measured, the study is reaching a stage of longevity in which it will begin to be appropriate to analyze the data for longitudinal trends and for intercorrelations of various related functions. In anticipation of this complex task the rate of conversion of data to a punch card system is being increased and preliminary tests of the data retrieval system have been conducted by the Human Performance Section.

These subjects, the Longitudinal Group, continue to provide a valuable cross-sectional population for a variety of studies by several sections of the Gerontology Branch.

Because of their high level of education, these subjects are of special value in studies of psychological functions. The Section on Experimental Psychology has been especially interested in determining the factors which lie behind the impaired learning ability which has been observed even in these subjects. Although the elderly subjects can learn material to the same criterion as the young, it takes them longer to do so. It is now apparent that the extent of the impairment observed depends on the conditions imposed in the learning experiment. In fact, when old subjects are allowed as much time as they want to respond they can reach the criterion of learning with the same number of trials as the young, but the time will be longer. The number of errors (as well as the number of trials required) increase more with age when the time given to respond is short than when it is long. In fact, the relationship between errors and age is curvilinear when the anticipation time is short, whereas it is linear with a smaller slope when the anticipation interval is long. It has also been shown that old subjects are more susceptible to interference (increase in errors) from extraneous material introduced between trials than are the young. However, it has now been found that interference is greater in old subjects than in young only if the time available to respond when learning the original task is short; if that time is long, there is no age difference in the degree of interference from an interpolated task.

These studies lead to the hypothesis that short-term memory storage may be a primary factor in the difficulties older people experience in learning. The experiments have shown, however, that alterations in the conditions of learning can compensate for this difficulty. It is further hypothesized that active responding is conducive to learning, particularly for an old person and lengthening the anticipation interval increases the probability of a correct response. It is also possible that re-enforcement by presentation of simultaneous cues from different sense modalities will enhance learning in the adult. Further studies to test these hypotheses will be

conducted.

Unravelling of the complex relation between (1) the decline in performance of a physiological function with increasing age, and (2) the increasing prevalence with age of a disease associated with that function is one of the major areas of investigation in clinical gerontology. A prototype of this problem is under study in the Metabolism Section: (1) the physiological decline with age in the ability to metabolize administered glucose loads, and (2) the age-associated increase in the prevalence of overt diabetes mellitus. The immediate goals of this program have been (a) to delineate the underlying mechanism(s) of the decline in function with age, and (b) to develop the first realistic age-adjusted standards for the several diagnostic tests for diabetes mellitus used in clinical medicine.

Studies on the Longitudinal Group had previously shown that the standards in common use for interpretation of the intravenous glucose tolerance test and the cortisone glucose tolerance test were appropriate for young adults only since their application to middle-aged and older adults resulted in a diagnosis of diabetes in 50% of the group. In the past year an extensive study was done on the Intravenous Tolbutamide Response Test as well. The test is an interesting addition to the diabetes diagnostic armamentarium of the physician since it is not simply a variant of glucose tolerance testing and reputedly has higher specificity than other diagnostic procedures. Results show that just as diabetics respond deficiently in the test, the presumably non-diabetic middle-aged and older longitudinal subjects also have deficient responses when compared to young adults. The rigid application of the currently recommended standards for normality to subjects over age 40 results in the classification of almost half of them as "diabetic". These unrealistic standards are probably the result of an inappropriate technique for selection of the subjects used to establish the normal standards for the test. From the results on the Longitudinal Subjects new standards for the test have been developed. These are presented in the form of a nomogram which permits the determination of the percentile rank of each individual among his age peers.

The Longitudinal Subjects have also been used as a group for the testing of one of the current hypotheses concerning the cause of decreased glucose tolerance. The hypothesis states that free fatty acids (FFA) in plasma inhibit glucose utilization. The decline in tolerance to glucose with increasing age then could be explained by an increasing concentration in FFA with age. Results in Longitudinal Subjects who were given oral glucose tolerance tests, however, show no correlation between FFA concentration and age or between FFA concentration and tolerance to glucose.

Results of a computer analysis of detailed 7-day food intake diaries on 252 Longitudinal Subjects have been analyzed under the supervision of the Nutritional Biochemistry Section. This again is purely a cross-sectional study at this time. The conversion of dietary food portions to the nutrient elements (calories, carbohydrates, protein, fat, minerals, and vitamins) was accomplished with a computer program devised by members of the Heart Disease Control Program. At the moment, it is possible only to characterize the nutrient intake of the Longitudinal Group as a function of age;

important correlations with other parameters (anthropometrical, physiological, and biochemical) will be the next step in the analysis of the data. Thus far it is clear that in the upper middle-class highly educated group of men, total caloric intake, vitamin and mineral intake tend to decrease with age with the main exception that intake of vitamin C does not follow this trend and, in fact, its intake is increased in the diet after age 70. Of interest is the fact that National Research Council recommended allowances were exceeded for all nutrients except Ca in the great majority of subjects. Calcium intake was below NRC allowances in about half of the middle-aged and older subjects. The pattern of food intake shows distinct differences with increasing age in that while the percentage of total calories derived from protein remains constant, more of the calories are derived from carbohydrate while fat plays a decreasing role as a source of calories. Whether these differences in caloric source in individual subjects on spontaneous diets will correlate with such parameters as serum lipid levels, carbohydrate tolerance, or the prevalence of atherosclerotic disease at the time of the study or its incidence in the future are some of the problems that remain to be determined.

Studies on the Longitudinal Group by the Pulmonary Physiology Section have begun to be examined for longitudinal trends in individual subjects. Preliminary analyses on 70 subjects who have had 4 visits show that with regard to vital capacity and maximal breathing capacity, only the older subjects show a downward trend in function while younger subjects show either no change or an increase in function. The ability to detect age differences in time trends in the relatively brief period of 6 years (4 visits) is encouraging.

A subsample of the Longitudinal Group, 20 subjects aged 39-83 years, has been studied in a set of experiments using techniques too complex to apply to the entire group. The characteristics of the barrier in the lungs across which gases must diffuse have been thought to change with increasing age; pulmonary diffusing capacity presumably decreases. However, these measured changes could be artefactual due to an increasing degree of non-uniformity of lung ventilation associated with aging. This hypothesis was tested using a technique for measuring pulmonary diffusing capacity by sequential steady state and washout methods. The extreme complexity of the equations generated by a multi-compartment system with non-uniform ventilation volume/diffusion was handled with the aid of a computer program (IBM 7094, University of Chicago). Thus more accurate measurements of diffusing capacity could be made in the non-uniform lung. These measurements show that part if not all of the age changes in diffusing capacity previously reported may be the result of an age differential in the non-uniformity error of the methods employed to make the measurement.

Future studies from the Section on Pulmonary Physiology will capitalize further on the advantages of the high speed digital computer in terms of accuracy, ease, and rapidity of the necessarily complex calculations in this field. In addition, pulmonary evaluations will be continued with measurements of lung volumes, pulmonary gas distribution, diffusion and compliance measurements. The addition of a body plethysmograph will also permit more direct estimates of the effects of deep breathing on thoracic gas volume and distribution.

New tests introduced into the longitudinal testing program in the past year included (1) detailed blood typing in order to characterize the participants immunogenetically, (2) "tapping-in-place" tests in order to investigate the possibility that the extra time required by older subjects in the two target tapping test is due to uncertainty in aim, and (3) a self-administered activity survey in place of the time-consuming interview.

Renal function in the Longitudinal Group has been evaluated with the 24-hour creatinine clearance test. In the past year the number of subjects with 3 consecutive tests has increased to 192, a group large enough to permit a preliminary examination for cross-sectional data, individual longitudinal trends, and within-subject variance. The cross-sectional data were best fitted with a second order regression; this development is interesting since, unlike most linear physiological age differences, in this test the decrements with age are small in early adult life, but become much more pronounced as age advances. The within-subject coefficients of variation at all ages are about 11-13 percent. The importance of this type of analysis is that it permits us to plan the most efficient testing schedule in terms of number and frequency of re-tests for the detection of longitudinal trends. Such information should also prove of value to other prospective studies on man now in progress or planned for the future. We plan to use similar analytical techniques for the other tested physiological variables in our program.

A number of clinical investigations in the Branch are being conducted on subjects other than the Longitudinal Group. In some of these studies the stimulus to the study again was the apparent similarity between changes occurring with advancing age on the one hand and in certain disease states on the other.

The Renal Section has continued its studies on the factors controlling urinary cation excretion. It had previously been noted that under conditions of a water diuresis during the basal state older subjects commonly (7 of 15 studied) had an inappropriately high loss of sodium in the urine. Unchecked, this defect could lead to hyponatremia. A detailed investigation has now been made of the mechanism of this defect and, in a related study, of the mechanism of the development of hyponatremia seen in certain acute and chronic illnesses (tuberculosis, malignancies, febrile states). For these comparative studies relatively healthy older subjects on the Gerontology Ward at the Baltimore City Hospitals composed the first group and all of the verified cases of hyponatremia (sodium concentration less than 125 mEq./L.) seen on the wards of the Baltimore City Hospitals during an 18-month period comprised the second group. The 16 hyponatremics were supplemented by another equal sized group of patients with similar illnesses, patients who might be expected to develop hyponatremia but whose serum sodium concentration had not yet reached abnormal levels. Distinct differences in these groups were found. The defect in the old but healthy group with inappropriate urine sodium loss was correctible by administration of glucose or related metabolites. Since the renal medulla is totally dependent upon glycolysis, this result suggests that an overnight fast leads to inadequate substrate supply to renal tubules with resultant inadequate sodium reabsorption; glucose availability then corrects this defect. In contrast, the acutely and chronically ill

patients and hyponatremics rarely showed this defect. In 4 carefully studied patients the syndrome of inappropriate anti-diuretic hormone secretion could be shown to be responsible for the hyponatremia. These subjects had not only balance studies, but also measurements of aldosterone secretion rates, steroid levels, blood volume determinations, and erythrocyte electrolyte levels.

In the past year another defect of the renal function in the aged has been reinvestigated. The observation had been made in the Gerontology Branch a number of years ago that recovery of serum pH following an administered acid load (ammonium chloride) was slower in older than in younger subjects. The mechanism of this defect has now been investigated by measuring the urinary excretion of "titratable acid" and of ammonium ion. Both of these avenues of H^+ loss in the urine were decreased in the older subjects. The decrease in titratable acidity was commensurate with the lowered glomerular filtration rate in the aged, but the diminished ability to excrete ammonium was proportionately greater than the GFR decrease. A further study was therefore conducted to test the hypothesis that the defective ability to excrete ammonia is due either to inadequate substrate (glutamine) or enzyme (glutaminase) for its production. Glutamine administration to old and young subjects resulted in equal increases in NH_3 production during an acid load and therefore the hypothesis seems unlikely.

An extension of these acid-base studies conducted by the Renal Section concerned the regulation of hydrogen ion concentration with the mitochondria of rat liver. Mitochondrial preparations met the criteria of respiratory control and their pH was measured with the C^{14} DMO technique. The pH was found to be linearly related to the extramitochondrial pH when the latter was set at levels of 6.90 to 7.60, but intramitochondrial fluid always remained somewhat more alkaline by about 0.3 pH unit.

The Endocrinology Section has also conducted studies in an area in which the change in a physiological variable (blood pressure) with increasing age resembles the change occurring in a disease syndrome (hypertension). Understanding of the relationbetween aging and disease in this instance again requires knowledge of the basic mechanisms underlying the changes. An intensive effort has therefore continued to develop a physico-chemical method for the measurement of plasma renin and angiotensin. Progress in this area of research has been long hampered by lack of such methods; heretofore, all analytical techniques depended on bioassays, most of which are of questionable specificity and precision. Over the past year we have succeeded in developing a double isotope derivative assay for angiotensin. This assay permits quantitative determination of nanogram amounts of the hormone. This degree of sensitivity is required for the measurement of the small quantities of angiotensin generated during in vitro assays of plasma renin. It should soon be possible to apply this new technique to the determination of renin. The importance of this project is along both theoretical and practical lines. Along theoretical lines, it is the first isotope derivative assay to be developed for a small polypeptide hormone and could lead to the development of other assays of similar important peptides. A practical renin assay, on the other hand, would be a major step in the everyday hospital differential diagnosis of

hypertensive disease, since it would almost certainly permit recognition of unilateral ("ischemic") renovascular hypertension. Recently the determination of plasma renin has assumed great importance in the diagnosis of primary aldosteronism, a disease which may account for as much as 10-25 percent of cases of hypertension heretofore considered to be of the "essential" variety. The problem has, therefore, a place in the attack on hypertension, a major age related disease complex. In addition, renin and angiotensin are intimately related to the secretion of the adrenal glands' mineralocorticoid hormone, aldosterone, and adequate methodology is badly needed in this area of physiology and pathophysiology. An additional aspect of the program is an interrelated study of renin-angiotensin-aldosterone in normal old persons. The data obtained in this study will establish normal standards for aldosterone secretion rate under conditions of salt loading and salt depletion. This information is unavailable but is badly needed in evaluating the role of aldosterone in the elderly hypertensive subject.

Another area of major interest to the Endocrinology Section is the field of thyroid hormone metabolism. The control of the rate of thyroid hormone degradation is age-dependent, as we have shown previously, but factors which determine this rate are largely unknown. By studying, as we presently are doing, the situation in acute febrile illness (bacterial infection) where rapid changes in hormone metabolism are known to occur, we hope to learn more of the normal control mechanisms for thyroid hormone metabolism and ultimately those involved in the changes with age. Of current interest are the interrelationships of hormone degradation rate, plasma thyroxine-binding proteins and plasma "free" (non-protein-bound) thyroxine. Preliminary results in human subjects experimentally infected with the organism of tularemia (Pasteurella tularensis) indicate that in this particular febrile illness thyroxine degradation rate is not accelerated, in contrast to the situation in other bacterial pneumonias. Nonetheless, thyroxine binding pre-albumin falls to low levels and, presumably, "free" thyroxine rises as a consequence. These results would appear to exclude "free" thyroxine and febrile illness, per se, as important physiological determinants of thyroxine degradation rate and will require extensive revision of current thinking in this area.

The remarkable but unexplained decrease in glucose tolerance with increasing age has provided the stimulus to the development of a new experimental technique for the study of the physiology of the glucose-insulin system by the Metabolism Section. The feed-back principle is used to adjust periodically the rate of infusion of glucose intravenously in order to maintain a predetermined new steady-state arterial glucose concentration. In these studies the hyperglycemic stimulus to the beta cells of the islets of Langerhans is constant and nearly identical in all subjects, in contrast to the marked variability in glucose levels that occur during the usual glucose tolerance tests. The ability to achieve a steady state with this technique is shown by the low coefficients of variation in glucose concentration over a two-hour period. In 32 subjects the mean CV was 3.9 percent. The time course of insulin release in response to sustained hyperglucemia is complex and cannot be described by a simple dose-response curve; in young adult subjects plasma concentrations of insulin continue to increase over the two-hour period. This changing insulin level is reflected by the progressively increasing glucose infusion rate needed

simply to maintain the arterial glucose concentration at its predetermined level. Older subjects differ from younger subjects in that (1) they require a lower glucose infusion rate and (2) they show less change in infusion-rate over the two-hour period. The implication of these age differences is that there is a diminished ability to respond to hyperglycemic stress in old age. The ability to increase plasma insulin concentrations progressively over a two-hour period which young people possess seems to be deficient in older subjects. These age differences raise questions concerning the mechanism of insulin synthesis and release in response to hyperglycemia; the servo-infusion technique should help to answer these.

BIOLOGY OF AGING

Although the quantitative description of age changes is of great importance, the development of methods for minimizing the deleterious effects of these changes depends on understanding the basic mechanisms of aging. This requires studies on an animal species in which genetic and environmental factors can be controlled and experimental techniques can be applied to isolated systems. Studies on basic cellular and molecular mechanisms must be pursued agressively. It is for this reason that part of the resources of the Branch have been devoted to studies of cellular biochemistry and the structural characteristics of molecules involved in biological processes.

Recent gerontological theory has attempted to explain age differences in motivation on the basis of physiological factors and in terms of differences in response to various environmental factors. A general factor termed "rigidity" has also been proposed as an explanatory construct. The program of the Section on Animal Behavior is concerned with (a) determining basic behavioral age differences, and (b) finding environmental factors which reduce age differences. The use of lower organisms such as the rat is necessary for such a program.

It has been found that environmental variables such as systematic handling by the investigator or testing during the dark portion of the dark-light cycle which reduced fear in young rats as evidenced by increased exploratory behavior generally had no effect on old animals. However, restriction of food intake increased exploratory behavior in both young and old animals. Thus, an increase in motivation (food deprivation) has resulted in behaviorally less rigid groups of animals, which are similar to animals younger in age.

An initial experimental study of taste discrimination was concerned with ingestion of very low concentrations of sucrose or quinine and the effects of past experience with quinine upon sucrose ingestion and vice versa for male rats 1, 5, 15, and 25 months old . Old animals did not discriminate the solutions as well as younger groups, and preceding ingestion experience was important (e.g., rats initially tested with sucrose drank more quinine and rats initially tested with quinine drank less sucrose). However the effects of past experience were clearly not a function of age, but rather of reduced sensory discrimination with increasing age. Changes in the plasticity of the

nervous system could be responsible for the reduction of sensory discriminability with increasing age.

A second experiment examined the ability of rats 1, 5, 15 and 25 months old to discriminate between two above threshold sucrose solutions. Normally rats prefer the more highly concentrated of two sucrose solutions. By making the discrimination more difficult, i.e., pairing very similar solutions, it was possible to examine age differences in the ability to detect the more highly concentrated solution. If the neural system of the aged rat was more rigid (e.g., reverberating cell assemblies were less flexible in form or resistant to change) than in the young rat, such discriminations should be more difficult for the older rats than for young. The data were consistent with such a hypothesis. In addition, when presented with palatable solutions young rats markedly increased their fluid intake, while senescent (25-mo.-old) rats were unable to alter ingestion rates. The fluid intake increase for young rats was greater as the discrimination became more difficult, suggesting that younger rats may also make more comparisons between tube pairs, i.e., have a higher general behavioral plasticity, than senescent rats.

Other experiments are planned to test further the hypothesis that aging is associated with greater rigidity of behavior. In this connection conditioning studies will be carried out on old and young animals with various schedules of training and the introduction of multiple responses.

Rats have also been used for studies on age differences in the performance of the isolated heart by the Cardiovascular Section. Additional experiments have fully confirmed the preliminary report that the heart of the senescent rat shows a significant impairment in its capacity to do work. The left ventricular work index expressed in gm-M/100 mg dry heart weight for the isolated heart-lung preparation was 34 ± 4 for 24-mo.-old animals and 166 ± 14 for 12-mo.-old animals ($P < 0.01$). Similar differences (15 ± 5 and 77 ± 13) were found for the isolated heart. It has also been found that direct electrical stimulation of the sino-auricular node fails to revive hearts that have worked to exhaustion. Hence it is concluded that the failure to perform is related to biochemical factors in the myocardium. These studies will continue to explore the biochemical mechanism of failure of the senescent hearts to perform as well as adult hearts. Studies of specific enzyme systems and concentrations of substrates essential for energy production will be conducted.

One of the current theories of aging proposes that with senescence alterations occur in the structure of the DNA molecule. This error is transmitted to messenger RNA and ultimately to newly synthesized enzymes. These defective enzymes may be inactive and therefore an accumulation of substrates within the cell may take place.

Data obtained during the past year in the Section on Nutritional Biochemistry have failed to support this theory of aging, which proposes an accumulation of structural errors in DNA which are reflected in RNA and protein, as well as increased turnover of these compounds to compensate for such errors. For example, it may be postulated that structurally altered

proteins should be detected by alterations in the mobility of enzymes during gel electrophoresis. However, no changes with age were found in the patterns of isoenzymes of seven dehydrogenases in various tissues of rats. Furthermore, although marked increases were found in the metabolism of RNA and protein when animals were fed ethionine to experimentally introduce errors into protein, a comparison of 40 old (24-31-mo.-old) and 40 young (12-mo.-old) rats failed to demonstrate differences in metabolism of RNA and protein between the two age groups.

It has been proposed that age changes may be most easily detected in enzymes which control specialized functions of highly differentiated cells. In order to test this proposal, two enzymes which are involved in neural conduction and transmission were measured in cerebrum, cerebellum and brain stem of young and old rats. However, no age-associated alterations were observed in the concentrations of DNA, RNA or protein or in the activities of acetyl cholinesterase or sodium-potassium activated ATPase.

Efforts made to identify the factors responsible for increased longevity in rats subjected to dietary restriction have required time consuming measurement of daily food allotments for large numbers of animals. During the past year, nutritional methods were sought which would avoid this necessity but would yield animals with similar characteristics and longevity. Preliminary data indicate that restriction of nutrients prior to weaning by (1) reduction of maternal intake or (2) employing, during lactation, mothers who had just nursed another litter, produced animals which exhibited low growth rates and which were unable to attain body weights equal to normal animals in spite of unlimited access to food following weaning. Although data on longevity are not yet available, these procedures may offer means of obtaining restricted animals without the laborious task of daily food rationing.

The experiments conducted thus far to test the error theory of aging based on the experimental introduction of structural alterations into protein were complicated by the influence of the ethyl group of ethionine on RNA and fat as well as protein metabolism. Therefore, studies will be conducted in which a different analog, viz., p-fluorophenylalanine, will be fed to rats and changes in RNA and protein metabolism will be measured in liver slices. In addition, the in vivo incorporation of radioactively labelled p-fluorophenylalanine and ethionine into liver proteins will be carried out to demonstrate that the analogs are incorporated into proteins. Finally, techniques are being perfected so that age-associated structural alterations in enzymes, as proposed by the error theory, can be detected by the quantitative determination of the isozymes of a variety of dehydrogenases in rat tissues.

The studies in progress to test the proposal that age changes may be most easily detected in those enzymes which control specialized functions of highly differentiated cells will be continued. Similar data to that already reported for the rat will be obtained on mice, viz., the activities of acetyl cholinesterase and sodium-potassium activated ATPase will be measured in cerebrum, cerebellum and brain stem and samples of these same sections will be examined for morphological alterations with age.

Efforts will be continued to obtain restricted rats without the laborious task of daily food rationing by restriction of nutrients prior to weaning. Studies will be conducted to produce large numbers of animals in order that data on longevity as well as information on the biochemical characteristics of such animals throughout the life span will be obtained.

In view of the key role of DNA and RNA in relation to "error" theories of aging the Gerontology Branch has placed special emphasis on studies of the molecular structure of these and related compounds.

The principal activity in the Section on Molecular Biology in the past few years has been the elucidation of the interaction of metal ions with the nucleic acids. There have been two main objectives in these studies: (1) to provide chemical techniques for the determination of the sequence of the nucleotides in the nucleic acid chains and (2) to understand the biological role of such metal ion-nucleic acid interaction. The importance of such fundamental studies to an ultimate understanding of the aging phenomenon is obvious.

During the past year the important discovery has been made that under appropriate conditions the degradation of RNA by metal ions such as zinc can be made specific, and this specificity can be changed by inhibition of the degradation by other metal ions. Thus the way has been paved for the use of nonenzymatic reactions in the specific cleavage of nucleic acids for sequence determination.

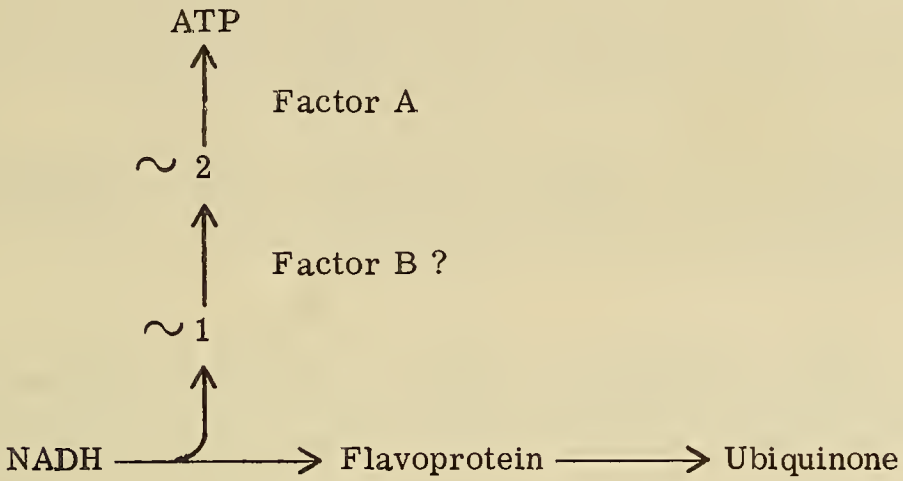
In the effort to determine the biological role of the metal-nucleic acid interaction we have carried out extensive investigations on the effect of metals on the reaction of DNA with polylysine, and the DNA-polylysine reaction itself has been subjected to intensive study. Several physical chemical techniques have been pioneered for the study of nucleic acid-protein interaction; the lack of progress in this important field has been due primarily to the absence of such techniques. Each nucleotide of DNA is bound to one amino acid in polylysine. The DNA-polylysine complex is severed by some metal ions and not by others. We believe that the unwinding and rewinding of DNA during replication involves such equilibria between nucleic acid, protein (histone) and metal ions. These studies do not prove this hypothesis, but represent the beginning of an attempt to unravel these complicated relationships.

During the coming year we intend to exploit the findings on the specific cleavage of RNA by metals. The reaction of metals with nucleic acids and proteins will lead to studies on the DNA-histone interaction, and, hopefully, to an understanding of the role of metals and histones in DNA regulation and the implication of these roles to the aging process. A new project on immunological studies on age-induced changes in tissue composition will be begun.

Previous work from the Section on Comparative Biochemistry had demonstrated that the oxidation of acetate-2-^{14}C to $^{14}CO_2$ occurred more rapidly in senescent than in adult rats. The findings have been explained on the basis of a change with age in the metabolic relationship between respiratory reactions of mitochondria and the extra-mitochondrial biosynthetic

reactions. In order to devise rational experiments to understand the molecular basis for the above observation, a detailed knowledge of cellular energy-linked reactions and their metabolic control is necessary.

The studies on the mechanism of oxidative phosphorylation in beef heart mitochondria have been extended. The work has centered on the first site of phosphorylation; viz., that occurring during the oxidation of NADH by ubiquinone catalyzed by the ubiquinone reductase flavoprotein. The experimental approach and findings are summarized in the following formal scheme.



A coupling factor, an enzyme tentatively called Factor A, has been purified further. Over 90% of the protein appears as a single band in electrophoresis on cellulose acetate or polyacrylamide gel. It restores P/O and related partial reactions in urea-treated-phosphorylation-deficient particles. These particles have only electron transport capacity in the absence of added Factor A. The purified factor has little ATPase activity, which can be stimulated over 20-fold by exposure to pH 8.5 and 43° C. The appearance of the ATPase activity is believed to be due to distortion of the active site concerned with the normal phosphate transfer function. The distortion could make the enzymephosphate complex susceptible to hydrolysis by increasing the accessibility of water.

The major accomplishment in the laboratory has been the isolation of a second coupling factor. The new factor, tentatively designated Factor B, is another heat-labile, non-dialyzable protein. In particles depleted of phosphorylation coupling factors by urea-treatment, Factor B above gave little stimulation of energy-linked reactions. In the presence of saturation levels of Factor A, however, Factor B gave large additional stimulation. In ammonia-treated particles, which respond but little to Factor A, Factor B produced extensive stimulation of ATP-driven NAD reduction and P/O. The results clearly establish that the two factors are distinct from each other. Factor B did not affect oxidation rates in any of the particles. Its role, very likely in the energy transfer sequence, remains to be investigated.

The flavoprotein in the above scheme is probably NADH-ubiquinone reductase. This enzyme has been isolated as two separate components with quite similar properties by chromatography on hydroxylapatite. It is conceivable that the two forms are derived from the same original mitochondrial segment by cleavage in two different ways. Both reductase preparations (QR1 and QR2) catalyze the oxidation of NADH by suspensions of ubiquinone-10 at high rates. They appear essentially as single, but separate, bands in electrophoresis on cellulose acetate strips and polyacrylamide gel. In sedimentation velocity experiments (carried out in collaboration with Dr. H. Edelhoch, NIAMD) better than 90% of the protein migrated under one peak. The molecular weights of QR1 and QR2 have been determined by sedimentation equilibrium to be 69,000 and 87,000 respectively. The reductases have bound FMN, nonheme iron and acid-labile sulfur which can be liberated as H_2S.

Thus, three of the components of the first site of mitochondrial oxidative phosphorylation system have been isolated. Indirect evidence has suggested the involvement of an additional undiscovered factor, structural protein and phospholipid in the over-all reaction.

Among the demonstrated changes occurring during aging is the loss of cells from a number of tissues containing irreplacable cell types (e.g., nerve cells). However, the events leading to cell death and loss during aging are not well understood and therefore during the past years fundamental studies on histochemical and chemical changes during cell death in model systems, under controlled conditions, have been carried out. Earlier histochemical studies on the ischemic death of cardiac cells carried out in the Section on Cellular and Comparative Physiology had indicated that irreversible injury occurs when heart muscle is kept under anaerobic conditions for sufficient periods to exhaust glycogen reserves and suggested that the irreversible phase is reached as the adenosine triphosphate concentration drops in consequence of the depletion of suitable glycolytic substrates. This thesis was tested using the cellular level of adenosine triphosphate as a function of the duration of anaerobiosis. It was found that ATP concentration drops to 50% of the initial level within 7 minutes after the imposition of anaerobic conditions and to about 5% of the normal level by 15 minutes. This decrease in ATP level was paralleled, surprisingly, by the loss of ability by isolated mitochondria to carry out phosphorylation reactions in vitro using the firefly continuous oxidative phosphorylation assay developed in this laboratory as the monitoring system. A study of the properties of these mitochondria revealed that they, as well as all mammalian and avian mitochondria tested, are extremely sensitive to dilution. Dilution below about 0.1 mg. mitochondrial protein/ml completely abolishes the capacity to phosphorylate adenine nucleotides. However, oxidative phosphorylation can be completely and instantaneously restored by the addition of about 0.5 mg/ml of serum albumin to the reaction mixture. With the exception of lactalbumin, which is about 15% as effective as serum albumin, no other proteins tested show this recoupling effect. This powerful influence of a serum protein on the efficiency of substrate utilization in the production of usable energy carrying intermediates suggests that these substances play a regulatory role in vivo. The level of serum albumin intracellularly, which in turn may be under hormonal influence (thyroxin, etc.), may thus moderate the oxygen consumption of tissue cells and the organism, and decreases during aging in the intracellular

concentration of serum albumin or analogous factors may lead to a decreased efficiency of production of usable energy intermediates such as ATP. The mechanism of serum albumin action in vitro will be examined, particularly its effect on mitochondrial myokinase (which in preliminary studies appears to be stimulated by SA addition). The intracellular level of serum albumin vs. age as well as other studies suggested by the above will be pursued further.

A variety of lines of evidence indicate that cessation of cell division as well as related differentiation processes play a central role in the aging of higher forms of life. For example, animals with indeterminate growth throughout their life span also frequently have indeterminate life spans (e.g., plaice, Testudo); cells in tissue culture appear to have a limited growth potential (about 50 cell divisions); underfeeding of experimental animals slows down both the growth rate and the rate of aging as measured by age specific mortality; and finally, a general inhibition of cell division is observable when differentiation takes place which results in many cases in cells which can no longer be replaced (e.g., nerve, muscle), which cells are also the locus of many functional decreases during aging.

In order to evaluate the role of cell division, arrest of mitosis and differentiation in the aging process, a deeper understanding of related cell biology is desirable and the research activities of cell biologists in the Branch and in the collaborating VA Aging Research Laboratory in Baltimore are directed toward questions bearing on the relationships between aging and these cellular properties.

Cell division in metazoa is influenced both by intracellular and extracellular factors. The finding by cell culturists (e.g., Hayflick) that there is a limited division potential in human cells in artificial media suggests a model of aging in which important cell lines may exhaust their division potential during the life of an individual and directly cause senescence. That this is a general property of cells capable of growing in culture has been shown during the past year. Chick myoblast cultures are capable of undergoing about 20 divisions and the number of divisions obtained decreases with increasing age of the donor embryo. Using human epidermis labelled with tritiated thymidine, supplied by Dr. Gerald Weinstein of the University of Miami, work in this laboratory has shown that this tissue in vivo is not subject to the limitation of fibroblasts in culture. This was established by radioautographically following the fate in serial sections of daughter cells of individual cells pulse labeled with tritiated thymidine. If the system follows the Hayflick rule, all daughter cells should move upward into the spinous layer coherently in groups of 2^n. Since single labelled spinous cells as well as triplets and other unpredicted groupings were observed in this human material, it appears that human epidermis is not limited to 50 divisions. Whether other important cell types are limited by this rule is the subject of present and projected studies.

Environmental factors affecting cell division and differentiation under controlled conditions constitute another facet of the research of the Cell Biology Group within the Branch. It has been shown that slight variations in the pH of the medium inhibit cell division and promote differentiation into

striated multinucleate muscle fibers. Below pH 7.2 cell division predominates; above pH 7.4 differentiation is favored. Interestingly, another factor which affects cell division is the level of serum albumin in the medium. Although some albumin is necessary for growth, somewhat higher levels are inhibitory. Related studies on the effect of sera of different ages, a la Carrell, are also under way.

Finally, the hypothesis that the process of differentiation leads to senescence by shutting off the synthesis of certain important cell components as a by-product of the specialized synthesis implicit in differentiation is being tested. These limiting components are supposedly long lived and possess a low turnover rate or no turnover at all.

The specific hypothesis being examined is that the pattern of synthesis is controlled by the species of s-RNA present and/or activated in specific differentiating cells. Since each amino acid is specified on the average by any one of 3 different codon triplets, the selection during differentiation of an appropriate group of triplets (codon set) for synthesis and/or amino acid activation would impose severe restrictions on the kinds of messenger that can be translated, since only those messengers can be read for which the corresponding species of s-RNA are available.

This proposition is being tested inter alia by comparing the pattern of s-RNA activation produced by activating enzymes from a variety of tissues from the same species. Identical s-RNA's are briefly labelled with tritiated amino acid in the presence of one enzyme and C^{14} labelled amino acid by another enzyme. The labelled s-Rna's are combined, chromatographically resolved and the ratio of tritium to C^{14} in each fraction determined. Concurrently the concentration of various s-RNA species present in different tissues is being examined.

Connective tissue is a fibrous matrix in which cells, water, mineral, vasculature, etc. are suspended. This idealized separation facilitates measurement of certain biophysical properties, viz., mechanics of deformation, diffusion-permeation and binding of water, the synthesis and depletion of macromolecular components in the matrix. Aging of connective tissue will be understood in terms of these properties, and others, as well as molecular and regulatory systems of fibroblasts and other tissue cells. Until there is a unified concept of the integrated connective tissue system, this artificial separation serves a useful prupose.

Present work in the Biophysics Section is concerned with the fibrous macromolecules of the connective tissue matrix, viz., collagen, elastin, and mucopolysaccharide. Since these substances perform a mechanical function in the physiological systems, the main research effort so far has been concerned with deformation analysis. The mechanics of tissue deformation was investigated in aging tendon, skin, and blood vessels. Experimental data on tendons suggests that longitudinal elasticity depends upon waviness of collagen fibers. A model of connective tissue elasticity was proposed in which the longitudinal extensibility is expressed as a function of the number of curled fibers which are transformed into uncurled fibers. Mathematical

representation of this model results in equations which are in agreement with experimental data. From this it appears that stiffness of large fibers increases during growth-development, but there is no further increase throughout the later period of life. On the other hand, small tendons show a concommitant reduction in stiffness; at all ages there is a negative relationship between stiffness and size of tendon (cross-sectional area). Since the original data were corrected for cross-sectional area, this negative dependence is a second-order effect. Presumably it represents an accumulation of imperfections during the build-up of large fibers. Aging, therefore, reduces the imperfections in large tendons, but it increases them in small tendons.

Data on aging dermis showed an initial linear extensibility, but this became non-linear at a critical strain. Morphological evidence suggests that the initial linear portion is due to elastin, whereas the non-linear data result from collagen fibers which become uncurled with stretch. Mathematical description of these delineated segments yields parameters of elasticity for aging dermis. It was noted that indices changed rapidly only during the early growth-development period of life. Comparison of these measurements with data in the literature also showed that composition of dermis, i.e., mucopolysaccharide, water, collagen, and elastin do not change rapidly during aging, but only during growth and development.

When the elasticity data of blood vessels were treated like that of dermis, it was seen that human arterial segments changed markedly during aging, but only slightly during the period of growth and development. Rat tail arteries and veins, in contra-distinction to human arteries, behaved more like refractile tendons, i.e., they did not change greatly during the later months of the life span.

Thus, one sees that connective tissue has both sensitivity and selectivity toward factors governing transformations during growth, development, and aging.

Small quantities of aged human skin can be obtained by dermatologic biopsy, but there are restrictions on the types of measurements possible because of the small size of the sample. Compression tests can be done on these small samples (biopsy), and data can be used to study molecular transformations of the fibrous matrix. It was observed that compressibility (stress-strain) data are similar to extensibility (stress-strain) data for calf and rat skin. This testing procedure will be expanded and used to study the biophysical properties of connective tissue in organs from which it might be possible to get only microscopic size tissue samples.

The above summary minimizes the importance of some observations which purport to show aging of connective tissue. This is true only to the extent that one recognizes the physical state of collagen in these samples. All of the above studies pertain to non-melted connective tissue, i.e., to collagen fibers which are in a state of high crystallinity. Biophysical analysis of elasticity of amorphous collagen in melted tendon or skin shows, in contradistinction with the crystalline state, that extensive changes are introduced at the molecular level. It is generally concluded that melted collagen chains

show an increase in the extent of inter-chain crosslinking, but recent evidence in the literature suggests a modification of this interpretation. It is now seen that perhaps these crosslinks are reoriented from intra- to inter-molecular tropocollagen, thus not requiring a priori the addition of many new crosslinks. This would provide a rational explanation for the limited increase in stiffness of crystalline collagen fibers in tendon and dermis. Vascular collagen, admittedly, would undergo more extensive crosslinking than tendon or dermis collagen, a case difference which will be investigated more thoroughly.

Endocrine systems appear to control or influence some parts of the deposition-polymerization mechanism of connective tissue. The extent to which this is modified by aging, or the possibility that it is the basis of changes in connective tissue with aging, now is the subject of speculation. A series of experiments now underway will delineate certain aspects of the control mechanism. Primary emphasis will be placed on the effect of age on the rate of response of connective tissue to an endocrine disturbance. This design is "longitudinal" in concept in that endocrine disturbances will be induced at various ages then followed by serial observation. The first series of measurements will determine the rate of response to hypophysectomy of rats which are hypophysectomized at various ages. Tests will be conducted on elasticity of dermis, tendon, and blood vessels to establish the generality, or specificity, of their response. Various organs will be weighed while the femur length and whole animal weight will be used to follow growth. Ultimately, the project will explore the longitudinal response to disturbances of pituitary, thyroid, and adrenal functions.

Serial No. HD-AG3(c)
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Longitudinal Studies of Human Physiology, Biochemistry and Psychology

Previous Serial Number: 185 (c)

Principal Investigators:
N. W. Shock (Director) (1/5 time)
A. H. Norris (Coordinator) (1/3 time)
R. Andres (Clinical Director) (1/8 time)
R. Swerdloff (1/3 time)
S. Adler (1/3 time)
J. Hollander (1/3 time)

Other Investigators:
M. J. Yiengst
C. H. Barrows
R. I. Gregerman
W. W. Surwillo
J. Tobin
H. Lutz
M. Schmukler
D. Arenberg

Cooperating Units:
Baltimore City Hospitals
Maryland State Health Department
Dr. Bernice Cohen, School of Hygiene and Public Health, The Johns Hopkins Univ., Baltimore, Md.
Mrs. Marjorie Zukel, Heart Disease Control Program, U. S. Public Health Service, Washington, D. C.
Dr. Robert B. McGandy, Department of Nutrition, Harvard School of Public Health, Boston, Mass.
Dr. Richard Feinberg, Georgetown Clinical Research Institute, Federal Aviation Agency, Georgetown University, Washington, D. C.
Dr. Robert Frisina, Gallaudet College, Washington, D. C.

Man Years
Total: 8.655
Professional: 1.656
Other: 6.999

Project Description:

Objectives: This project is designed to: (1) facilitate repeated measurements of various physiological, biochemical and psychological variables in the same individuals as they grow older, (2) coordinate the utilization of the results of these measurements in establishing indices of aging and (3) compare the effects of socio-economic status on these results and the indices which may be derived therefrom.

Methods Employed: Subjects are volunteers recruited by other participants. They agree to be available for a three-day series of tests every 18 months for the rest of their lives. A given series of tests will be repeated at a five year interval. Measurements of body size and composition, pulmonary function, kidney function, neuromuscular function and cardiovascular function are made. Standardized psychological tests include the Guilford-Zimmerman Temperament Survey, Southern California Tests of Creative-Thinking Abilities (Word Fluency, Associational Fluency I, Expressional Fluency, Ideational Fluency I, Consequences), Benton revised Visual Retention Tests and the vocabulary subtest of the Wechsler Adult Intelligence Scale. Also included are two verbal learning procedures of appropriate difficulty to provide a wide distribution of scores for the longitudinal subjects. Physical examinations and medical histories are performed with check lists to assure uniformity of recording. Medical findings and pertinent test results are reported to a participant's private physician only. Participants are treated as members of an elite group and kept informed of the progress of the total program and of test results for the group. Data are kept in restricted files and are summarized by calculating machine and electronic computer methods.

Major Findings: The sixth series of tests was initiated in April, 1966 for participants who had completed 5 series of tests. Five hundred and eighty-seven participants have been tested one time, 422 two times, 300 three times, 142 four times, 79 five times and 6 have been tested six times. Twenty-five participants have resigned or withdrawn from the program and 28 have died. This loss of 53 individuals represents 9 percent of the sample group. Two questionnaires designed to measure the attitudes of the longitudinal studies participants have been summarized. One which measures agreement with stereotypical statements about old people (Tuckman and Lorge, Attitude Toward Old People) was summarized for 421 participants. No relationship was found between the tendency to subscribe to stereotypical beliefs about old people and the chronological age of the participant. Tuckman and Lorge found that subjects who answered the question, "In your opinion, when does old age begin?" by specifying a chronological age subscribe more to stereotypes about older people than those who specify other factors. This tendency was not observed in this group of longitudinal studies participants. The second questionnaire (Burgess, Cavan and Havighurst, Your Activities and Attitudes.) has been summarized for 465 participants in the longitudinal studies. The scores revealed no significant relationship with chronological age in this group. The similarity of attitudes between old and young persons found here may reflect the atypical nature of the sample and may result, in part, from the high educational level of the participants (86% have college degrees).

Thus old and young participants in the longitudinal studies were found to be similar rather than different with respect to the activities and attitudes measured by these questionnaires. In addition, some demographic characteristics of the participants were derived from the Burgess, Cavan and Havighurst questionnaire. Ninety-eight percent of the group were born in the United States, 93% self-rated their health as good or excellent, 84% were in professional or managerial occupations, 92% were married and living with their spouses. Seventy-six percent of the participants professed a protestant religious faith and 12% stated that they were Roman Catholic.

Proposed Course of Project: Collection of this body of data will place us in a position to test theories of aging against the human population of the longitudinal studies. Results of comparisons between old and young individuals are presently available, but these do not permit us to evaluate theories of aging adequately. Our ability to do so will depend in part upon a comparison of the integrated medical, physiological, psychological and biochemical profiles of the individual participant with various subsequent outcomes which we may be in a position to monitor in the same individual. Outcomes such as length of life, occurrence of disease, rate of change in a functional parameter, among other things will be sought. Some theories assume that a few cells are lost during each unit of time so that in an individual or in an individual organ system changes of a gradual nature would be found. These theories are consistent with the summation of a series of distributed minor accidents (such as radiation hits) if only a few cells are involved each time. Other theories would relate large changes in function with the occurrence of a disease episode, or major accident resulting in a substantial loss of cells. These theories are consistent with abrupt changes in functional levels which would be maintained until another major accident results in a new level. In addition, these two widely different types of theories could be found in combination with sudden as well as slow changes operating simultaneously. Rapid access to these complex relationships in individual participants will be invaluable in reaching conclusions about the appropriateness of theoretical formulations for this population.

Honors and Awards:

1. Willard Thompson Award
2. Gerontological Society Research Award

Publications:

1. Shock, N. W.: Gerontology and geriatrics. In: Encyclopedia Britannica Encyclopedia Britannica, Inc., Chicago, 1965, pp. 363-365.

2. Shock, N. W., Andres, R., Landowne, M., Norris, A. H., Simonson, E., and Swartz, F. C.: Aging of the cardiovascular system. In: E. C. Andrus (Editor), The Heart and Circulation. Vol. I. Research. Federation of Amer. Soc. for Experimental Biology, Washington, D. C., 1965, pp. 558-584.

Serial No. HD-AG4(c)
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH

Project Title: Age Changes in Human Performance

Previous Serial Number: NHI-186 (c)

Principal Investigator: A. H. Norris (1/3 time)

Other Investigators: N. W. Shock

Cooperating Units: Baltimore City Hospitals
Dr. A. T. Welford, The Psychological Laboratory, University of Cambridge, Cambridge, England
Dr. Robert W. Ramsey, Medical College of Virginia Richmond, Virginia
Dr. John Magladery, The Johns Hopkins University, School of Medicine, Baltimore, Maryland
Dr. Robert D. Teasdall, The Johns Hopkins University, School of Medicine, Baltimore, Maryland

Man Years:

Total:	2.249
Professional:	.333
Others	1.916

Project Description:

Objectives: This project is designed to study the effects of aging on: (a) the physiologic responses to exercise, (b) the rate of recovery of physiologic equilibrium after exercise, (c) muscular efficiency and (d) work output and fatigue. In addition, the factors responsible for limitations in performance observed in older people will be evaluated.

Methods Employed: Measured amounts of physical work are obtained in subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake, CO_2 elimination, pulmonary ventilation volume, heart rate, blood pressure, and cardiac output (by the dye method) are taken before, during and after standardized amounts of exercise. In addition, the volume of the lungs and functional capacities of the pulmonary system are evaluated. Other studies include measurements of speed of nerve conduction, reflex delay time, and muscle action potentials. These phenomena are recorded on a six channel oscillograph or dual beam oscilloscope as the experiment demands.

Major Findings: Pulmonary spirometric measurements performed for the longitudinal studies participants have been reviewed. In addition, two methods for the determination of Residual Volume have been compared. Residual Volume has been determined by a helium washout technique as well as a nitrogen washout technique. The helium washout was performed with the participant in a recumbent position while the nitrogen washout was performed with the participant in the seated position. When a direct comparison of results made for 47 individuals who had both tests during the same visit to the laboratories, the nitrogen washout gave values which were higher than the values for the helium washout by the amount attributable to the postural difference (nitrogen, sitting; helium, recumbent). Thus, we have found these tests to be interchangeable for the measurement of Residual Volume. Changes in vital capacity and maximum breathing capacity have been sought in 70 participants who have had 4 visits for testing within a period of six years. We have made the simplifying assumption that trends for individuals are not different from straight lines and have calculated the linear regression coefficient of vital capacity and of maximum breathing capacity on age for each participant. A summary of the results by age decade groups shows that although the average slopes of regression were not different between old and young groups, the trend from positive slopes in the young groups to negative slopes in the elderly is clear. Thus, average decrements in function are suggested only in the old. While the large variability of slopes among individuals limits the usefulness of the slope as an index of change on an individual basis, the differences between groups in direction (sign) of mean slope is encouraging.

Proposed Course of Project: An integrated appraisal of participants in the longitudinal studies will be based on the exercise screening procedures and measurements of the cardiovascular and metabolic responses to exercise as well as measurements of tapping speed, nerve conduction velocities, and reaction and reflex latencies. Pulmonary evaluations will be continued with measurements of lung volumes, gas distribution in the lungs, lung diffusing capacities and compliance measurements which may account for differences in ventilation volume, breathing rate and lung volumes. In addition, we hope to obtain a body plethysmograph to make more direct estimates of the effects of deep breathing on thoracic gas volume and distribution in older persons.

Honors and Awards: None

Publications:

1. Mittman, C., Edelman, N. H., Norris, A. H., and Shock, N. W.: The relationship between chest wall and pulmonary compliance and age. J. Appl. Physiol., 20: (6), 1211-1216, Nov. 1965.

Serial No. HD-AG5(c)
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Age differences in body size and composition

Previous Serial Number: NHI - 187

Principal Investigator: A. H. Norris (1/3 time)

Other Investigators: N. W. Shock
M. J.Yiengst
R. I. Gregerman

Cooperating Units: The Baltimore City Hospitals
Dr. Harald Schraer, Department of Biophysics, Pennsylvania State University, University Park, Pennsylvania
Dr. Stanley M. Garn, Fels Research Institute, Antioch College, Yellow Springs, Ohio

Man Years
Total: .916
Professional: .333
Other: .583

Project Description:

Objectives: This project is designed to describe age differences in body size and composition measures made concurrently in individual subjects, and to examine the relationship of these age differences and comparisons to physiological responses.

Methods Employed: Height and weight are obtained by the usual anthropometric methods. The volume of the body is measured by its displacement of helium in a closed chamber. The density of the body is calculated from these measures after correction for total skeletal mass estimated from roentgenographic films of large and small bones. Body fat is also estimated from skinfold thickness and roentgenographic techniques. Estimates of indices of lean body mass include: (1) basal metabolic rate determinations made with standard open circuit methods, (2) urinary excretion of creatinine and (3) body water and fluid distribution determinations made from the distribution curves of injected

antipyrine and sodium thiocyanate. This is a continuing program. These studies are carried out in people of different ages and in the same people as they become older.

Major Findings: This measurement program has been continued in support of the longitudinal studies. Each participant had initial or repeat measurements according to a fixed schedule. One of the goals of this study is to compare changes in body composition with nutritional intake and physical activity levels. A comprehensive interview has been used in this laboratory to estimate the caloric value of average daily physical activity for 252 of the longitudinal studies participants. These values ranged from 2,500 to 5,000 calories per day for participants between 30 and 70 years of age while participants over 70 had estimated energy expenditures of 2,200 to 4,000 calories per day. Since this interview requires a large part of the testing time which is available, a self-administered questionnaire has been developed as a substitute. Direct comparison of results from the interview and from the questionnaire were made for 11 individuals. Differences between the two methods were less than 10% of total Calories/day in 9 individuals and 15% and 20% of total Calories/day in the remaining two individuals. Although the average Calories per day from the questionnaire (3512) was 270 Calories greater than the average estimated by interview (3242), this difference was not considered to be significant. The similarity of the two methods is further supported by the fact that the questionnaire gave higher values 6 times in the 11 comparisons and the interview gave higher values in the remaining 5 of the 11 comparisons.

Proposed Course of Project: We hope to determine whether the lower mass of tissue in persons over 70 is: (1) a terminal sign associated with disease or a change in nutrition or activity or (2) a result of making measurements in a selected group, the survivors. Individual patterns of bodily changes will be followed in the participants in the longitudinal studies to determine if the degree of tissue loss is related to survival. The integrated measurement program will be continued. In addition to the selected indices of body size and composition, we hope to introduce measurement of total body potassium or total exchangeable potassium to estimate lean body mass. Some techniques are provided through cooperation of investigators outside of the Public Health Service while others are standardized procedures used in this laboratory. Data are available for comparison with other physiological data which may be collected on subjects of these studies. Moreover, subjects whose size or composition varies widely from mean values will be selected for study, and experimental and therapeutic displacements of body composition may be attempted.

Honors, Awards, and Publications: None

Serial No. HD-AG6(c)
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Problem Solving and Age.

Previous Serial Number: NHI-199 (c)

Principal Investigator: D. Arenberg

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	.70
Professional:	.30
Other:	.40

Project Description:

Objectives: Until recently, most efforts to explore human problem solving have been focused on end-products such as correctness of a solution and time to reach a solution. The solution processes, however, are of far greater interest and value in understanding problem-solving behavior. One reason for slow progress in this direction has been the paucity of methods which provide observable solution behaviors which can be objectively measured. Recent attempts to develop such methods have been fruitful, and two of these are described below and are used in this project.

One general objective is to determine in what ways age is related to problem-solving performance. Are age differences a function of difficulty or type of problem? If age differences are found, they can be explored further. For example, the differences may be due to difficulties of older people in acquiring the relevant information; or perhaps the differences are a function of the utilization of information (synthesizing acquired information). Are solution strategy-preferences related to age? Even if no age differences are found in the end results, there may be important age differences in how solutions are reached. The experiments in this project are designed to answer such questions.

Methods Employed: A modification of the Problem Solving and Information Apparatus (PSI) developed by John at Chicago is used in Experiment I. Each problem is displayed on a board with ten lights, nine of which have buttons to turn them on. The task of the subject is to light the light which has

no button (the goal light) and this must be done in a prescribed way. There is a disk with a pattern of arrows for each problem. An arrow indicates that one of three logical relations (previously explained to the subject) exists between the lights connected by the arrows, but the specific relation is not known. Part of the problem is to determine the meanings of the arrows by inference or by questions of a prescribed form. The subject is instructed to infer as much information as he can in solving the problem. The ultimate solution of each problem is lighting the goal light by a sequence of inputs in which only a specified set of three of the nine buttons can be used. Time between inputs is controlled by the subject.

There are many dependent measures possible for each problem in addition to whether the subject arrives at a solution and how many questions he requires. Two such measures are (1) the number of questions required for all relevant information to be known or inferable, and (2) the number of questions needed for all relevant information to have been asked. Other information type measures can be extracted such as the number or proportion of questions asked about information inferrable from the pool of information available at the time the question was asked. (This pool of information changes during solution.) This is one measure of redundancy, but others can be defined such as the number or proportion of questions or sequences of questions which are repeated.

Experiment I consists of four test problems increasing in difficulty preceded by a demonstration problem and a practice problem. The test problems are sufficiently challenging to provide a wide range of performances among the (high-level) subjects in the longitudinal program. Comparable forms of these problems can be constructed for repeat administrations.

Concept-attainment problems are also included in this project. The task in such problems is to identify the attributes which define a subset of all elements in a set which consists of all possible combinations of attributes. For example, if every element has one of three colors, one of three numbers, and one of three figures, there are a total of 27 elements. If the subset consists of all elements with one specified color, then the 9 elements with that color are members of the subset. By asking for or being given the information that specific elements are or are not members of a subset, the subject must determine the concept, i.e., the attribute(s) which define the subset.

For the simple concept problems used thus far, each element is provided by the experimenter and identified as a member or non-member of the concept subset. After each element is identified, the subject is required to report the concepts compatible with all the information available. Performance measures are derived from comparisons of the subject's report with the correct set of concepts at each point in the solution.

Experiment II was a pilot study of concept attainment using ten simple problems consisting of two comparable sets of five problems. The five pro-

blems include: (1) all elements positive, none redundant; (2) all elements positive, one redundant; (3) all elements negative, none redundant; (4) positive and negative elements, none redundant; and (5) positive and negative elements, one positive and one negative redundant. Although the concepts were simple (a color, a number, or a design), the instructions were cumbersome (due to the abstractness of the task) and required several practice problems. As a result, many of the solutions were apparently obtained without reasoning. To avoid this undesirable result, procedural modifications were needed which would substantially reduce the number of practice problems.

A reduction in the number of practice problems was achieved by changing the task, making it much more meaningful and concrete, but without changing the essential logic or information content of the ten problems. In Experiment III, the subject no longer attempts to identify which of nine attributes (colors, numbers, designs) is the concept. Instead he is required to identify which of nine foods has been poisoned. Elements are now meals of three foods rather than cards with a color, number, and design. Positive elements are meals which result in death (rather than "Yes" cards); negative elements are meals which do not result in death (rather than "No" cards). Although the problems are logically identical to those in Experiment II, the instructions are far less cumbersome because the task is readily understood. Only one practice problem is needed. Successful solutions appear to be reached by reasoning processes. Furthermore, fewer subjects are now excluded due to failure to understand instructions.

Major Findings: Process measures have been developed for Experiment I (logical problem solving), and the performances of the more than 250 Ss available are currently being scored using the new measures. Each response in a solution is classified in one of four mutually exclusive classes of response: (1) new information uninferable from the current information pool; (2) information indirectly inferable from the current information pool (involving a chain of reasoning); (3) information directly inferable; (4) overtly redundant. Data collection continues as part of the longitudinal program.

At the time this report was written, valid performances of 21 old and 13 young men were available in Experiment III (concept attainment). A comparison of the two age groups resulted in a clearcut difference favoring the young group in number of correct solutions. Despite the fact that the problems are simple concept attainment tasks presented as real-life problems, and despite the average intelligence of the Ss, few of the men over 60 were able to solve more than half of the problems. In the young group, only three of the thirteen men solved fewer than seven of the ten problems. An additional 32 old Ss were given the same set of problems, but an oral rather than a written report of the concepts (foods) compatible with all the information available was required. These solutions have not yet been scored.

Proposed Course of Project: Data collection will continue for Experiment I, and age analyses will be made of the process measures. In Experiment III,

data collection will be completed for the young group. The kinds of problems which gave old Ss particular difficulty and the types of errors which were most prevalent among the old group will be explored and compared with those of the young group. Both Experiment I and Experiment III will be prepared for publication. Conjunctive and disjunctive problems may be included in subsequent experiments.

Serial No. HD-AG7(c)
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Verbal learning and age.

Previous Serial Number: NHI-197 (c)

Principal Investigator: David Arenberg (60% time)

Other Investigators: None

Cooperative Unit: Baltimore City Hospitals

Man Years:

Total:	1.20
Professional	.60
Other:	.60

Project Description:

Objectives: The broad objectives involve determining factors which affect age differences in verbal learning to provide experimental evidence for explanations of age deficits. Immediate objectives are to determine interactions with age resulting from varying several time intervals and to evaluate the effects of inter-item interference, active responding, and various combinations of auditory and visual inputs on acquisition and retention measures.

Methods Employed: In Experiment I, 348 longitudinal Ss between 20 and 87 years of age were randomly assigned to one of two anticipation intervals in a serial-learning task of twelve familiar, five letter nouns. The same Ss learned a paired-associate task at one of two anticipation intervals in Experiment II. Each of the eight items consisted of two consonants serving as the stimulus elements and a familiar, two-syllable adjective as the response element. Total errors and trials-to-criterion (one errorless trial) were the dependent measures, and orthogonal polynomial analyses were applied to these measures at each anticipation interval for each task. These analyses provided comparisons of linear and quadratic components of the age curves underlying the interaction between age and anticipation interval previously found when data for young and old groups were included in an analysis of variance.

In Experiment VIII, the paired-associate method was used with two age groups and three pace variables. The anticipation interval, the inspection interval,

and the inter-item interval were varied using the number of frames in five different films projected at a constant speed to provide accurate and precise timing of all intervals.

In Experiment X, a prompting condition was compared with the typical confirmation condition. Prompting refers to a procedure in which the inspection interval precedes the anticipation interval, the reverse of the usual order. This procedure increases the probability of active responding (correctly) during acquisition trials. The timing of the intervals and display of the material was accomplished by a bank of five slide projectors controlled by a cam timer. In the self-paced test trials, one of the projectors was advanced by a manual switch. Each of six acquisition trials, consisting of twenty-four paired items, was followed by a self-paced test trial. Two hours after the final test trial, a recall measure of retention was obtained using two more self-paced test trials. No feedback is provided on test trials.

Experiment XI was a retroaction study of paired-associate learning with age and anticipation interval the independent variables. The eight items from Experiment II were used for original learning and relearning. The eight items of the interpolated task consisted of the same eight pairs of consonants and eight,two-syllable adjectives unrelated to the first eight words. Each task was learned to a criterion of one errorless trial. Two anticipation intervals were used: 1.9 sec. and 3.7 sec. The inspection interval (1.9 sec.) and the inter-item interval (1.8 sec.) were constant. A card-changing device was used to present the material and to control all time intervals. Two age groups (30-39 and 63-77 years) were included. The 48 Ss were participants in the Branch longitudinal program and were randomly assigned to one of the two anticipation intervals for all three tasks (original, interpolated, and relearning).

Experiment XII was a short-term retention study with three input conditions: (1) visual only, (2) visual and auditory, and (3) saying the material aloud as it appeared. A film of consonant sequences and digit sequences was prepared to provide 32 test items, 6 practice items, and 4 screening items at each of the three conditions. Each digit and each consonant was shown for 0.50 sec. (12 frames). The screening items consisted of a four-digit sequence and a short interval before writing the response. These items were used to determine whether the S could retain four digits when no interfering material followed. The practice items consisted of a three-consonant sequence followed by a two-second interval and another three-consonant sequence. The second group of consonants were said aloud by the S as they appeared; they served as interfering stimuli. A S was instructed to write the three consonants in the first group after saying the second group of consonants. The test items were similar to the practice items, with the exception that the first group consisted of a four-digit sequence and the second group consisted of a three-digit sequence. The three input conditions depended upon what occurred when the first group of digits or letters appeared. In the visual-only condition, the S looked at the first group as

each element appeared. In the visual-with-auditory condition, the experimenter said each element of the first group aloud while the S looked at each element on the screen. In the condition with visual input and active responding, the S said each element of the first group aloud as the element appeared on the screen. Each S performed in all three conditions. A 16 mm movie projector externally modified with a monitoring synchronous motor provided precise timing of the visual displays. A decade interval-timer was used to start and stop the projector to show a constant segment of film for each item.

Major Findings: Linear components were evident in all eight orthogonal polynomial analyses of data from Experiments I and II, and the slopes of the linear regressions of performance on age were steeper at the short than at the long anticipation interval for both the serial and the paired-associate tasks using either errors or trials-to-criterion measures. Quadratic components were evident only for the four analyses involving the short anticipation interval (both measures at both tasks); no quadratic components emerged in the four analyses involving the long anticipation interval. Therefore, the interaction found previously between age and anticipation interval can be attributed both to steeper linear slopes at the short than at the long interval and also to positively accelerated curvilinearity at the short interval. These studies have been prepared for publication in one paper.

One of the findings of Experiment VIII (in which three pace variables were included) was that the age difference was smaller with no inter-item interval than with a two-second inter-item interval. This was contrary to the general hypothesis that the faster the pace, the greater the age difference; therefore, a replication of this aspect of Experiment VIII was initiated and is in progress.

The hypothesis that learning is improved for an old person when he actively responds was tested in Experiment X. In the confirmation condition during acquisition trials with a short anticipation interval, an old person makes few responses. In the prompting condition, the correct word is seen immediately before the S is supposed to respond. The old S was expected to respond much more frequently in prompting than in confirmation. If the "active responding" hypothesis is correct, and more responses are made during acquisition trials in prompting than in confirmation, a learning difference favoring the prompting condition should result for old Ss. It was also hypothesized that the age difference would be smaller in prompting than in confirmation. Data collection was initiated for Experiment X.

Experiment XI was an attempt to reconcile the contradictory findings of the two retroaction studies of verbal learning and age in the literature. These two earlier studies differed considerably in anticipation interval. If the anticipation interval is related to retroaction age differences as it is to age differences in learning, the contradictory findings of the earlier studies are reconcilable. In Experiment XI, this hypothesis was confirmed.

An age difference in retroaction was found at the short but not at the long interval, and the difference between age differences at the two intervals was statistically significant. This study has been submitted for publication.

It was recently reported that age differences in short-term retention were greater for visual than for auditory input of the material to be retained. This result may have implications concerning the "active responding" hypothesis mentioned above. An old person may learn and retain material more effectively when he responds than when he does not because he hears his response. To explore this possibility, two conditions of auditory input were included in Experiment XII. In one condition, the material (digits or letters) was spoken by the experimenter and the S saw and heard it; in the other, the S said the material aloud as he saw it. If active responding is no more than a vehicle for auditory input, performance in the two auditory conditions should be equivalent, and both should be superior to the visual-only condition. If, on the other hand, active responding is more than a way to achieve auditory input, then performance when the S says the material should be superior both to passive auditory input and to visual without auditory input. Data collection has been initiated for Experiment XII.

Proposed Course of Project: Data collection will continue for Experiments VIII, X, and XII, and each will be prepared for publication.

Publications:

Arenberg, D.: Anticipation interval and age differences in verbal learning. J. Abnorm. Psychol. 70: 419-425, December, 1965.

Arenberg, D., and Surwillo, W. W.: A device for paced and self-paced visual displays. Amer. J. Psychol. 79: 120-123 , March, 1966.

Serial No. HD-AG8(c)
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The relations between age and time estimation measures of accuracy, variability, and context effects.

Previous Serial Number: NHI-198 (c)

Principal Investigator: D. Arenberg

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years:

Total:	.20
Professional:	.10
Other:	.10

Project Description:

Objectives: (1) To determine whether age is related to simple time estimation measures (accuracy, variability, and context effects). (2) To determine to what extent each of these four variables (age and the three time estimation measures) can account for performance on a more complex time judgment task involving interference from a set of interpolated stimuli. Similar judgment tasks have been found to be related to age in earlier work in this laboratory.

Methods Employed: Fourteen intervals on a log scale from 0.62 seconds to 2.14 seconds were used to make up two overlapping sets of eleven intervals each. One set was symmetrical about one second; the other set was predominantly above one second (two below, eight above, and one at one second). The subject's task was to indicate whether the interval a light was on seemed longer or shorter than one second. A total of 110 judgments (ten for each of the eleven intervals in the symmetrical set) provided a point of subjective second (PSS, a measure of accuracy) and a measure of intra-subject variability (discriminability). A PSS value was also obtained from the 110 judgments on the asymmetric set of intervals. Comparison of the two PSS values provided a measure of shift or context effects, i.e., how much the subjective second changed with the change in the distribution of stimuli.

The more complex time judgment task, which provided a measure of interference, has been presented in two ways. The first procedure required the

subject to discriminate four time periods (0.20, 0.35, 0.62, and 1.10 sec.) to a criterion, then to discriminate four intervals (1.10, 1.61, 2.14, 2.68 sec.) for twenty presentations (five of each interval), and finally to discriminate the original four intervals to the earlier criterion. Previous work had indicated that on this type of task, old subjects have more difficulty discriminating the four short periods after the interpolated judgments than do young subjects. The second procedure was the same as the first except for the number of intervals presented. A fixed number of stimuli was used for all subjects in the initial and final discrimination tasks instead of the variable number of stimuli based on each subject's performance. The measure of interference was based on the increase in the number of errors in judging the four short intervals following the interpolated stimuli.

Major Findings: Data collection was initiated using the new procedure for time judgment in which a fixed number of stimuli are presented to all Ss. Data for the 50 men over 60 had been collected but data for only 37 young men (17 to 21) were available at the time this report was written. No analyses have been made.

Proposed Course of Project: Data for the last 13 Ss will be collected and scoring will be completed. Correlational analyses similar to those made with the criterion measures will be calculated. Current plans are to include in one paper the results of the two major studies together with the methodological study of the new time-judgment procedure.

Honors and Awards:

Surwillo, W. W., and Arenberg, D.: On the law of initial value and the measurement of change. Psychophysiology 1: 368-370, April, 1965.

Serial No. HD-AG9
1. Gerontology Branch
2. Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Age differences in the control of plasma insulin concentration.

Previous Serial Number: NHI-164

Principal Investigators: R. Andres. (50% time)
R. Gregerman (5% time)
R. Swerdloff (32% time)
J. Tobin (65% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals
H. Langs, Department of Medicine, The Johns Hopkins Hospital

Man Years:

Total:	2.52
Professional:	1.52
Other:	1.00

Project Description:

Objectives: The long-range objective is to gain an understanding of the physiological factors controlling release of insulin by the beta cells of the islets of Langerhans, and more specifically, to test the hypothesis that the progressive decline in glucose tolerance with increasing age is secondary to a disturbance in the beta cell responses to hyperglycemia. An immediate goal is to measure plasma insulin responses in a series of tests of carbohydrate metabolism on subjects covering the entire adult age span of life (the Longitudinal Study Group of the Gerontology Branch). A second immediate goal is to devise a technique for the study in man of the glucose-insulin closed loop system which will permit close experimental control of the glucose concentration. The method would enable the investigator to set the glucose concentration at any desired level for any period of time and would permit comparison of beta cell response in subjects of diverse ages to identical steady-state hyperglycemic stresses. It would also provide for the first time an accurate dose —>response curve in man for the glucose —>insulin system.

Methods Employed: (1) Insulin is assayed by a partially automated modification of the Morgan and Lazerow double antibody radio-immunoassay. (2) Plasma samples are obtained during the studies described in the Project entitled "Age changes in carbohydrate metabolism in man" and from the servo-control studies described below. (3) Blood glucose servo-control studies are based upon (a) rapid analysis of glucose concentration in arterial blood by a modification of the ferricyanide-reduction methodology using the Auto-Analyzer, (b) continuous infusion of glucose intravenously using a continuously-variable infusion apparatus. Infusion rates during the initial 15 minutes of the study are determined by an empirical formula designed to raise the arterial glucose concentration within 2-4 minutes to a new predetermined steady-state concentration. The analysis time for glucose has been reduced to five minutes, so that at 15 minutes the glucose concentration of the 10 minute sample becomes available. Adjustments in glucose infusion rate are thereafter carried out at 5 minute intervals from 15 to 120 minutes. The formula for adjustment in infusion rate was devised to accomplish correction of the glucose concentration rapidly but to avoid overcorrection and the consequent danger of harmonic buildup of swings in glucose concentration. The servo-correction formula has two components. One adjusts the maintainance infusion rate in the appropriate direction. The second provides for the glucose needed simply to fill the glucose space with the required amount of glucose. The formulae are rather complex and will therefore not be detailed in this report. Despite the complexities the adjustment in infusion rate can be carried out within a 1-2 minute period with the use of a desk calculator.

Major Findings: Thirty-two servo-control studies have been performed. The technique is successful as judged by the constancy of arterial glucose levels achieved at 3 chosen concentrations: 140, 180, and 220 mg per 100 ml. This constancy, expressed as the coefficient of variation in concentration over a two hour period, averages 3.9 percent. Several conclusions are worthy of note: (1) Young adults require about twice as much glucose infusion as old subjects (in terms of mg glucose per kg body weight per min) at the glucose concentrations chosen. This result is another demonstration of the reduction in glucose tolerance with aging, and was to be expected. (2) There appears to be a linear increase in glucose infusion rate required over the range of concentration rates used in the young subjects; there is a tendency toward decreasing glucose need at the higher levels in older subjects, that is, glucose infusion rates in the 220 experiments were very little higher than those required to maintain a 180 concentration. (3) The glucose infusion rate following the initial priming period, in the young subjects increased with time despite the constancy of the hyperglycemic stimulus to the beta cells. There is a tendency toward a plateau period from 30 to 90 minutes followed by an increased need from 90 to 120 minutes especially with the studies carried out at 180 and 220 mg per 100 ml glucose concentrations. (4) Insulin assays have been completed in six of these studies. The only conclusion that should be drawn on these preliminary data is that the course of the insulin concentration verifies the pattern of the glucose infusion rates during the two hour studies. Thus

despite constancy of the hyperglycemic stimulus to the beta cells, the insulin concentration in arterial plasma increases progressively during the studies.

The insulin assays on the series of tests on the Longitudinal Study group are gradually being completed. Assays were done on 161 studies last year and on 178 studies this year. Effort this past year was aimed at completing the series of 150 tolbutamide response tests and this has been accomplished. The complexity of the analysis involved in studying the time course of the insulin response and in relating this to changes in glucose and free fatty acid concentrations will require the aid of a computer and this program is being developed.

Proposed Course of Project: The range of glucose concentrations achieved in the servo-control study will be increased to 300 mg per 100 ml at least. A series of studies will be done in which the continuous intravenous glucose infusion will be complicated by an oral glucose load at 60 minutes. The objective will be to continue to maintain the same arterial glucose concentration during the subsequent two hours. These studies are designed to test the hypothesis that there is a gastro-intestinal factor which is stimulated by oral glucose administration and which causes release of insulin independent of the stimulus provided by the glucose concentration in the environment of the beta cell. The technical proficiency of these studies will be further improved by using an electronic calculator which can be programmed to perform fairly complex calculations rapidly. The lag-time in adjustment of glucose infusion rate will thus be decreased considerably. Insulin assays on the experiments performed this past year will be completed.

Publications

1. Andres, R., Swerdloff, R., Pozefsky, T., and Coleman, R.: Manual feedback technique for the control of blood glucose concentration. In: Automation in Analytical Chemistry, 1965, Technicon Symposium, 1966.

Serial No. HD-AG10(c)
1. Gerontology Branch
2. Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Age changes in carbohydrate metabolism in man.

Previous Serial Number: NHI-195 (c)

Principal Investigators: R. Andres (25% time)
R. Swerdloff (25% time)
J. Tobin (25% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals
H. Langs, Department of Medicine, Johns Hopkins Hospital

Man Years

Total:	2.45
Professional:	0.75
Other:	1.70

Project Description:

Objectives: The prevalence of diabetes in all populations increases strikingly with age. The commonly accepted prevalence figures (derived from community surveys) range from a fraction of 1% in young adults to a peak of 6-8% in the later decades of life. In contrast to these values stand the results of glucose tolerance testing in aged population groups: about 50% of older subjects are "abnormal" when judged by the usual standards of normality for these tests. It is the purpose of the present studies (1) to resolve these discrepant results, (2) to define standards of performance on a variety of diabetes diagnostic tests in an active community-dwelling group of males covering the entire active adult age span, and (3) to investigate the reasons for the decline in tolerance with age in order to discover whether the physiological and pathological factors underlying the age effect are the same as those responsible for the decline in tolerance in the diabetic state.

Methods Employed: The schema described in the 1963-64 Annual Report is being followed with only minor modifications. Subjects are primarily from the Longitudinal Study Group supplemented by college student volunteers to increase the size of the 3rd decade group. All tests are performed under basal conditions using standardized quality control techniques.

Major Findings: In the past year 399 tests were added to the 488 performed in the previous two years. The emphasis in the past year has been on the intravenous tolbutamide response test. This test had been shown to be markedly influenced by the diabetic state and had furthermore been proposed as a more specific test for diabetes than the various versions of the glucose tolerance test. Several findings are of interest. Age has a profound influence on performance. Young adults show a rapid decline in glucose concentration which reaches a nadir in 30 minutes and rebounds toward the control value at 45 and 60 minutes. Middle-aged and old adults show a much more gradual fall; the curves resemble those described for known diabetics. The rigid application of the recommended standards for normality to subjects over 40 years of age results in the necessity to classify almost 50% of them as "diabetic". We have carefully considered the reason that such unrealistic standards are in use. A review of the 25 papers which provide original data for this test in conjunction with our own results shows that the factor responsible for the inappropriate standards is that of the technique of selection of test subjects. First, if young adults comprise the test series then standards appropriate for that age group only are derived. Secondly, some authors have set rigid pre-selection criteria for inclusion in the test series by demanding superior performance on preliminary glucose tolerance testing. Since glucose and tolbutamide tolerance are highly correlated, the mean tolbutamide response for a group of subjects can be artifically raised or lowered by having either high or low cut-off points on the preliminary glucose tolerance test.

The technique for judging performance on the tolbutamide test that we have developed rests upon the principle that subjects be judged against their age cohorts. Nomagrams have been constructed from our data on the tolbutamide test which permit the rapid simple determination of the percentile rank of each individual among his age peers. The decision as to which percentile rank at specific ages is to be considered as true diabetes is a moot one at the moment and will possibly require the passage of years in the prospective study to resolve.

The oral glucose tolerance test series was increased in the past year from 100 to 250 subjects. This larger series has permitted delineation of several additional facts. The correlation between glucose concentrations at 60 and at 120 minutes is only 0.68. The fact that the two values are correlated is expected, but the relatively low correlation coefficient casts doubt on the proposal that the glucose tolerance test be shortened to 60 minutes in place of the more usual two hour test. The effect of age on performance is also seen more clearly at 120 than at 60 minutes. Glucose concentration at 60 minutes increases by 4.4 mg per 100 ml per decade of life while at 120 minutes the increase is 6.4. The larger series also shows that variance in the results increases with age. This finding has an influence on the nomagram construction technique for this test. A useful nomagram for the oral test has now been prepared.

Progress has been made in the analyses for free fatty acids (FFA) on the

plasma samples obtained during the test procedures. In the past year 364 sets were analyzed. Preliminary analysis of the results in the oral glucose tolerance test series shows that there is no correlation between age and the fasting FFA level. There is furthermore no effect of either the fasting FFA level or the level at 60 minutes on glucose concentrations during the test. We have therefore obtained no evidence that the FFA concentration is an important physiological determinant of performance on the glucose tolerance tests, as has been recently proposed.

Publications:

1. Andres, R.: Aging, glucose tolerance, and diabetes - a proposal. In Gitman, L. (Ed.): The Endocrines and Aging. Springfield, Ill. Charles C. Thomas, 1966.

Honors:

1. Dr. Andres was an invited speaker at the 1966 post-graduate course of the American Diabetes Association held at Washington, D. C., Jan. 19-21.

2. Dr. Andres is an invited participant for the Pre-Congress Colloquium on Research and Training for Clinical Gerontology at Semmering, Austria, June 23-25, 1966.

Serial No. HD-AG11(c)
1. Gerontology Branch
2. Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Effect of cortisone on carbohydrate metabolism in man.

Previous Serial Number: NHI-196 (c)

Principal Investigators: R. Andres (12.5% time)
R. Swerdloff (10% time)
J. Tobin (10% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	0.625
Professional	0.325
Other:	0.300

Project Description:

Objectives: Steroid-augmented glucose tolerance tests have been proposed as predictors for the development of the overt diabetic state. The rationale for this use is that while all subjects show a decreased tolerance to glucose after steroid administration, potential diabetics show an exaggerated response in this regard. Since aging also is accompanied by a decline in glucose tolerance, if this decrement were due to the increasing incidence of true diabetes, then the response to steroids should also become exaggerated with advancing age. To test this hypothesis the cortisone glucose tolerance test (CGTT) is being administered to subjects covering the adult age span in a prospective study.

Methods Employed: The technique described by Fajans and Conn was followed with only minor modifications. Subjects were members of the Longitudinal Study Group of the Gerontology Branch supplemented by college students and hospital and laboratory employees to increase the number of subjects in the third and fourth decades of life.

Major Findings: A detailed description of the effect of age on performance in the CGTT has been reported in the current year. Fifty-six additional subjects have been studied as part of the continuing longitudinal study program since that report. Subjects who on previous visits received the CGTT

have been given an oral glucose tolerance test (OGTT) on their return and vice versa. At the time of submission of the manuscript for publication, no OGTT had yet been performed by us. Now that 130 oral tests have been done, it is clear that the age effect on performance on the cortisone test is indeed much greater than it is on the oral test. For example, two hours after glucose administration the glucose concentration increases by 18 mg per 100 ml per decade of life on the cortisone test but increases by only 6 mg per 100 ml per decade on the oral test. This exaggeration of the decline in carbohydrate metabolism in the older subject by steroid administration is the same as that described for the younger subject who is destined to become diabetic. On the basis of this test then, the effect of aging is the same as that of an early stage in the development of the diabetic state.

Proposed Course of Project: The CGTT will continue to be employed as one of the battery of tests in the Longitudinal Study program. The usefulness of the test as a predictor of the overtly diabetic state must await the passage of years.

Publications:

Pozefsky, T., Colker, J. L., Langs, H. M., and Andres, R.: The cortisone-glucose tolerance test: influence of age on performance. Ann. Int. Med. 63: 988-1000, 1965.

Serial No. HD-AG12
1. Gerontology Branch
2. Nutritional Biochemistry
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Age Changes in Cellular and Tissue Biochemistry

Previous Serial Number: NHI - 168

Principal Investigators: C. H. Barrows, Jr. (1/4 time)
M. Schmukler (1/2 time)
R. E. Beauchene (1/2 time)
J. Hollander (2/3 time)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	4.416
Professional:	1.916
Other:	2.5

Project Description:

Objectives: The general purpose of this program is to examine various tissues for metabolic changes associated with senescence. Specific investigations during this period have included experiments to (1) study the effect of age on enzymatic activities of brain, (2) determine the effect of age on the pattern of isoenzymes in rat tissues and (3) study the effect of age on protein and nucleic acid metabolism in the rat.

Methods Employed: (1) Acetyl cholinesterase and sodium-potassium activated ATPase activities were measured by standard procedures in homogenates prepared from the cerebrum, cerebellum and brain stem from the right half of the brain in 15 month old and 28 month old female Wistar rats. In addition, the concentrations of DNA, RNA and protein were measured in these same preparations. Finally, similar preparations from the left half of each brain were paraffin sectioned and stained with H & E, Nissl, Bodian's Protarcol and Woelcke's Myelin stains in order to evaluate age-dependent changes in relative cell populations (neuron vs. glia) and to ascertain if the Alzheimer changes, observed in the human brain during senescence, could

be detected. (2) The qualitative patterns of the isoenzymes of lactic, malic, glucose-6-phosphate, isocitric, 6-phosphogluconic, α-glycerophosphate and alcohol dehydrogenases were determined in the brain, liver, kidney, heart and muscle of rats of different ages by acrilamide gel electrophoresis. (3) ^{14}C-leucine was used to estimate protein synthesis in liver slices as well as in a cell-free ribosomal system. RNA synthesis was estimated in liver slices by the incorporation of either ^{14}C-uridine or ^{14}C-orotic acid. The catabolism of protein and RNA were estimated by the activities of cathepsin and RNase respectively in liver homogenates.

Major Findings: (1) No changes in the concentrations of DNA, RNA or protein or in the activities of acetyl cholinesterase or either total or sodium-potassium activated adenosine triphosphatase as a function of age could be found. Only preliminary histologic observations have been made. No gross changes have been seen thus far. No change in brain weight was found. The intragroup variability is higher in the older than younger animals. (2) No qualitative changes with age in any of the isoenzyme patterns were found in the tissues examined. A technique for quantitation of the individual lactic dehydrogenase isoenzymes was developed using electrophoresis on cellulose polyacetate, followed by elution of the protein or determination of activity by the fluorometric assay previously reported. (3) Protein synthesis as measured in liver slices or in the cell free system was the same in old and adult rats. Catheptic activity was higher in the livers of the older than in the younger animals. However, no age-associated changes were found in uridine nor orotic acid incorporations into RNA of liver slices, RNase activity or the concentrations of protein, RNA, and DNA of liver homogenates.

Proposed Course of Project: Studies will be conducted to (1) establish whether or not there are quantitative differences in isoenzymes in rats and rotifers of different ages, (2) determine the glucose tolerance curves in rats of different ages and (3) determine the effect of age on the sulfhydryl content of plasma albumin.

Honor and Awards: None

Publications:

1. Beauchene, R. E., Fanestil, D. D., and Barrows, C. H., Jr.: The effect of age on active transport and sodium-potassium-activated-ATPase activity in renal tissues of rats. J. Geront. 20: 306-310, 1965.

2. Barrows, C. H., Jr.: Changes in enzyme levels and patterns. In Brues, A. M., and Sacher, G. A. (Eds.): Aging in Levels of Biological Organization. Chicago, Ill., University of Chicago Press, 1965, pp. 152-186.

3. Schmukler, M., and Barrows, C. H., Jr.: Age differences in lactic and malic dehydrogenases in the rat. J. Geront. 21: 109-111, 1966.

Serial No. HD-AG13
1. Gerontology Branch
2. Nutritional Biochemistry
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Aging Studies in the Rotifer

Previous Serial Number: NHI - 169

Principal Investigators: C. H. Barrows, Jr. (1/4 time)
M. Schmukler (1/2 time)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years
Total: 2.25
Professional: .75
Other: 1.5

Project Description:

Objectives: The general purpose of the program is to determine the physiological and biochemical characteristics of rotifers at various ages throughout their life span and to determine the basic biological mechanisms responsible for the influence of factors which alter longevity in these organisms.

Methods Employed: Two of the major problems in aging studies on rotifers are (1) the lack of rigidly controlled culture conditions due to inadequate knowledge of the nutritional requirements of the organism and (2) the difficulty of obtaining large numbers of animals of the same age due to contamination with newly hatched rotifers. These difficulties must be overcome in order that a variety of biochemical measurements can be carried out and in order to evaluate more precisely the influence of nutrition on life span. Efforts during the past year have been directed toward solving these problems by (1) evaluation of the use of various defined culture media and (2) development of techniques in which rotifers of known age can be obtained from mass culture.

Major Findings: Results obtained thus far indicate that neither the pond water nor the algae could be replaced by

Knapp's salt solution or powdered milk respectively without an appreciable (25%) decrement in the life span of rotifers, although the total egg production was unaffected by these substitutions. It was found that 10^{-4} M dinitrophenol, which had no effect on egg production, inhibited the development of eggs. This provided a means of separating the rotifers of known age from developing young organisms. During this period techniques were also developed for the detection of isoenzyme patterns of malic, isocitric and glucose-6-phosphate dehydrogenases in the rotifer.

Proposed Course of Project: The new techniques developed for harvesting rotifers will be used to investigate the effect of age on DNA, RNA, specific enzymatic activities as well as on the qualitative and quantitative patterns of isoenzymes.

Honors and Awards: C. H. Barrows - Elected chairman of Biology Section, Gerontological Society

Publications:

Fanestil, D. D., and Barrows, C. H., Jr.: Aging in the rotifer. J. Geront. 20: 462-469, 1965.

Serial No. HD-AG14(c)
1. Gerontology Branch
2. Nutritional Biochemistry
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Human nutrition studies: nutrition survey in the longitudinal study

Previous Serial Number: NHI - 166 (c)

Principal Investigator: C. H. Barrows, Jr. (1/4 time)

Other Investigators: N. W. Shock
A. H. Norris

Cooperating Units: Maryland State Health Department
M. Cantoni, A. Spanias, and A. Meredith
Heart Disease Control Program, USPHS
Washington, D. C.
Baltimore City Hospitals
R. B. McGandy
Harvard School of Public Health

Man Years

Total:	.25
Professional:	.25
Other:	0

Project Description:

Objectives: (1) To characterize dietary intakes and patterns of eating in individuals in the Longitudinal Study Group and to identify possible age-related differences between individuals. (2) To correlate age-related differences in nutrient intake with age-related difference in various anthropometrical, physiological and biochemical parameters. (3) By a repeat survey in five years, to describe individual changes in dietary intake as a function of time and to relate these to alterations in laboratory measurements on the same individuals.

Methods Employed: For the purpose of surveying the dietary intakes and eating patterns, 7-day diet record forms have been kept by the subjects in the Longitudinal Study who have had the initial medical screening examination. To assess seasonal variability, three such records were collected on each man

during the year until January 1, 1965. Subjects who began participation after this date will keep only one diet record per year. A 3-day record of food intake is used as a source of instruction while the subject is in the hospital. The nutritionist and physician accompany the subject to lunch and discuss estimated portions and method of recording. For possible assessment of changes in past food habits, two short questionnaires are used. The data thus collected will be analyzed for the purpose of characterizing the types of foods eaten as well as for quantitation of specific nutrients by the methods in use at the National Heart Institute. Correlations between nutrient data and information already being collected in this study on such parameters as body composition, estimates of basal energy expenditures and plasma cholesterol and hemoglobin levels will be performed.

Major Findings: The coded data from the first and second dietary records of 252 subjects have been processed by computer analysis. The results showed a marked age-associated decline in total daily caloric intake. Similar decrements were observed in the vitamin and mineral intakes with the exceptions of that of vitamin A, which declined only between the ages of 20 and 50 and of vitamin C, which showed no change with age except for high values in subjects aged 70. In general, with the possible exception of calcium, the nutrient allowances suggested by the National Research Council were exceeded by a great majority of the subjects. Although the percent of total calories derived from protein was constant with age, those from fat declined markedly after age 60. Finally, the age-dependent decrement in total caloric intake could not be ascribed solely to a decrease in basal calories.

Proposed Course of Project: The manuscript reporting this work is now in preparation.

Honor and Awards: None

Publications: None

Serial No. HD-AG15
1. Gerontology Branch
2. Nutritional Biochemistry
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The relationship of nutrition to biological aging

Previous Serial Number: NHI - 167

Principal Investigators: C. H. Barrows, Jr. (1/4 time)
R. E. Beauchene (1/2 time)

Other Investigators: R. L. Davis
B. F. Chow

Cooperating Units: Baltimore City Hospitals
V. A. Hospital, Bay Pines, Florida
Johns Hopkins University School of Hygiene & Public Health

Man Years

Total:	1.75
Professional:	.75
Other:	1.0

Project Description:

Objectives: The general purpose of this program is to investigate the influence of nutrition on the rate of biological aging as estimated by changes in various biochemical parameters in tissues of the rat. Specific investigations during this period have included studies to (1) test the hypothesis that, with advancing age, errors occur in DNA molecules which are transmitted to RNA templates and ultimately to protein molecules, (2) determine whether the vitamin B_{12} marginal deficiency states observed in older people are associated with hematological and biochemical changes, and (3) determine the extent of influence of limitation in food intake during lactation in the rat.

Methods Employed: (1) a. Male and female adult rats were fed Purina Laboratory Chow containing either 1/8% or 1/16% ethionine for periods of time up to 2 months. At that time the animals were sacrificed and the liver removed. Liver slices were employed to study protein metabolism using ^{14}C -Leucine as a tracer; ^{14}C-Leucine was also used to study

protein synthesis in a cell free ribosomal system. RNA metabolism was followed using liver slices and ^{14}C-Uridine as a tracer. In some experiments ^{14}C-orotic acid was used to follow RNA metabolism. Cathepsin and RNase was determined on liver homogenates. b. An analogous experiment was performed using p-fluorophenylalanine, an analog of phenylalamine, added to the basic diet at levels of 0.08% and 0.16%. (2) Since hemoglobin and hematocrit values as well as lactic acid levels and the activities of lactic dehydrogenase in sera have been shown to be influenced by vitamin B_{12} deficiency in humans and in rats, attempts were made to correlate vitamin B_{12} levels with these variables in 573 ambulatory male domiciliary members of a VA center. (3) Two experimental procedures were used to limit the food intake of rats during lactation. In the first, the dietary intake of the mother rat was reduced 50% during gestation and lactation. The livers and kidneys of the progeny, as well as of normal animals, were analyzed for the activities of D-amino acid oxidase, succinoxidase and alkaline phosphatase by standard procedures. In the second, the young were given to mothers who had nursed another litter just prior to this time. The rates of disappearance of glucose from the blood following subcutaneous injection in these animals were compared to those of normal rats.

Major Findings: (1) a. ^{14}C-Leucine incorporation into protein was significantly greater in ethionine-fed animals than in controls, regardless of sex, but in female rats the ratio was increased. This was true using either liver slices or the ribosomal system. Uridine incorporation into liver RNA of ethionine-fed animals was not different from that of control animals. Neither liver cathepsin nor liver RNase showed significant directional changes. However, liver RNA concentrations were markedly increased in the ethionine-fed rats.

The increase in liver RNA concentration with ethionine-feeding for either 1 or 2 month time periods suggested that either the incorporation of ^{14}C-Uridine into liver slices was not adequately sensitive to demonstrate differences in the synthesis of RNA or that increased synthesis of RNA had occurred prior to the end of the 1-month feeding period. Therefore, a second study was carried out in which the animals were fed ethionine for 1 week. The results indicated a marked increased rate of incorporation of ^{14}C-orotic acid, but not of ^{14}C-Uridine into liver slice RNA.

(b) Fluorophenylaline -- While there was a tendency for fluorophenylalanine-fed animals to incorporate greater amounts of leucine into protein of liver slices, none of the differences were statistically significant. There were no statistically

significant differences in any of the other systems tested either.

(2) Although regression analyses performed on samples obtained from the 5th through the 9th decades of domiciliary subjects for two successive years indicated a decrement in serum vitamin B_{12} level with age no significant changes with age were found in lactic acid level, lactic dehydrogenase activity or hematocrit values, nor any significant correlations between these variables and vitamin B_{12} levels.

(3) Under both experimental conditions of reduced food intake during lactation, the growth of the progeny was stunted, even though the animals were allowed unlimited access to food after weaning. For example, approximately one year after birth, the mean body weights of rats nursed by normal and foster mothers were 525 gm. and 419 gm. respectively for males and 403 gm. and 317 gm. respectively for females. Due to the limited number of animals available and the need for further information on growth rates and longevity under these experimental conditions, only a few rats have been sacrificed for biochemical studies. Although no marked differences were found in the activities of the various enzymes measured there was a tendency toward higher values of alkaline phosphatase activity in the kidneys of rats born of restricted than of normal mothers. This finding is similar to the results obtained on normal rats subjected to a 50% reduction in food intake at weaning. Data thus far obtained indicate that rats restricted during lactation exhibit an abnormal glucose metabolism as evidenced by higher plasma levels following injection but slower rates of disappearance than found in normal animals.

Proposed Course of Project: Studies will be conducted to (1) further evaluate the effects of undernutrition during lactation on longevity and various biochemical characteristics such as the concentrations of DNA, RNA, protein and the activities of enzymes, (2) determine whether the rate of cell proliferation is reduced in animals subjected to reduced dietary intake by measuring the *in vivo* incorporation of H^3-thymidine, (3) determine the nitrogen balance in rats prior to and during the terminal loss in body weight.

Honor and Awards: None

Publications:

Barrows, C. H., Jr., Roeder, L. M., and Fanestil, D. D.: The effect of restriction of total dietary intake and protein intake and fasting interval on the biochemical composition of rat tissues. J. Geront. 20: 375-379, 1965.

Serial No. HD-AG16(c)
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1963 through June 30, 1966

Project Title: Evaluation of hypotensive therapies in the treatment of essential hypertension.

Previous Serial Number: NHI-191 (c)

Principal Investigator: R. D. Lindeman (12 1/2% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals
The Johns Hopkins University School of Medicine
C. R. Cooke
R. P. Russell
F. Wolff

Man Years:

Total:	.125
Professional	.125
Other	none

Project Description:

Objectives: Outpatients in the BCH hypertension clinic have been utilized to study (1) the value of established hypotensive therapy in reducing morbidity and mortality in hypertensives, and (2) the effectiveness of some of the newer hypotensive therapies. To learn more about the mechanisms and sites of action of ethocrynic acid, a potent new saluretic agent, a series of studies, including stop flow and tissue slice analyses, were conducted in dogs.

Methods Employed: The results of a double-blind study comparing the effects on morbidity and mortality of drug therapy vs. placebo in a group of patients with mild to moderately severe hypertensions has been reported in the previous annual report.

The potent saluretic agent, ethocrynic acid, was subjected to a clinical trial as an antihypertensive agent as reported in the last annual report. A series of studies were conducted in dogs to investigate the mechanisms and sites of action of ethocrynic acid.

Major Findings: The results of these three studies were covered in the last annual report.

Proposed Course of Project: The manuscript for the clinical trial with ethocrynic acid is being completed. No further studies are contemplated.

Publications:

Cooke, C. R., and Lindeman, R. D.: The site of action of ethacrynic acid and its effect on the renal cortico-medullary sodium gradient. Bull. Johns Hopkins Hosp., 117: (5), 271-285, Nov. 1965.

Wolff, F., and Lindeman, R. D.: Effectiveness of antihypertensive therapy-Results of a controlled study. Journal of Chronic Diseases, , March, 1966.

Serial No. HD-AG17(c)
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Renal response to suboptimal infusions of antidiuretic hormone. Influence of age, renal disease, hypertension, diuretics and calcium.

Previous Serial Number: NHI-189 (c)

Principal Investigators: R. D. Lindeman (1/8 time)
M. J. Yiengst (1/8 time)

Other Investigators: N. W. Shock

Cooperating Unit: Baltimore City Hospitals

Man Years:

Total	.25
Professional	.25
Other	none

Project Description:

Objectives: The initial study designed to determine the relative ability of a fixed amount of vasopression given by a constant infusion (8 milliunits per hour) to alter the renal antidiuretic response under a variety of conditions (age, renal disease, hypertension, diuretic therapy and calcium infusion) was completed. One of the major problems in the study was that certain assumptions had to be made about the rate of clearance of ADH from the human circulation based on animal studies. An attempt will be made to determine in humans more about the relative importance of the kidney and liver in the clearance of ADH.

Methods Employed: An attempt was made to develop an ADH bioassay in the rat.

Major Findings: It was possible to measure known amounts of ADH standard down to 2 to 4 microunits in .2 ml samples. Contaminants in untreated plasma interfered with the determinations. Problems relating to collection and preparation of the plasma samples remained to be resolved.

Proposed Course of Project: Six months ago, this study was shelved when it was found that it could not be completed before the principal investigator was scheduled to leave the unit. Efforts were redirected to complete several other projects.

Publications:

Lindeman, R. D., Lee, T. D., Jr., Yiengst, M. J., and Shock, N. W.: Renal response to suboptimal infusions of antidiuretic hormone. Influence of age, renal disease, hypertension, diuretics and calcium. Accepted for publication. J. Lab. Clin. Med., August, 1966.

Lee, T. T., Lindeman, R. D., Yiengst, M. J., and Shock, N. W.: Influence of age on the cardiovascular and renal responses to tilting. J. appl. Phy-Physiol., 21: (1), 55-61, Jan. 1966.

Lindeman, R. D., Sear, R. O., and Lee, T. D., Jr.: Hand-Schuller-Christian disease with occult diabetes insipidus. Arch. inter. Med., 116: ( ), 131-137, July 1965.

Lindeman, R. D.: "Glomerulonephritis" In: H. F., Conn (Editor), Current Therapy 1966. W. B. Saunders Co., Philadelphia, 1966, pp. 402-409.

Serial No. HD-AG18(c)
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on the inter-relationships between carbohydrate and intermediary metabolism in the human kidney and the urinary excretion of electrolytes.

Previous Serial Number: NHI-188 (c)

Principal Investigators: R. D. Lindeman (1/2 time)
S. Adler (1/3 time)
M. J. Yiengst (1/8 time)

Other Investigators: N. W. Shock
C. R. Cooke

Cooperating Units: Baltimore City Hospitals
Johns Hopkins School of Medicine, Department of Medicine

Man Years

Total:	1.955
Professional:	.955
Other	1.00

Project Description:

Objectives: An attempt has been made to define some of the inter-relationships between renal metabolism of various nutrients (ethanol, glucose, galactose, fructose, xylose, protein and fat) and the renal tubular transport of electrolytes (sodium, potassium, calcium and magnesium).

Methods Employed: Healthy male volunteers, age 19-93 years have been utilized in these studies. Following an overnight fast, each subject received 20 cc of water per Kg of body weight and was allowed to reach a near maximum diuresis. Urines were collected during three 20 minute control periods and urine volumes replaced by equal amounts of fluids given orally or intravenously. At this point, some form of treatment was instituted. Four subjects ingested on successive tests 100 grams of glucose, 100 grams of galactose and nothing (control). Urines were collected during four subsequent 20-minute periods. Bloods were drawn at the midpoints of the 1st, 3rd, 5th, and 7th periods. Determinations of glucose, galactose, lactate pyruvate, total organic acids (urine only), sodium, potassium, calcium, magnesium, chloride, phosphate, uric acid, inulin and PAH were made on each sample.

Additional studies were conducted under similar conditions except that creatinine clearances replaced inulin and PAH clearances and studies were carried out for three additional 20-minute periods or more. Subjects received variable amounts of glucose (25 to 100 grams) intravenously and orally to compare effect of route of administration dose-response relationships and duration of effect. Other subjects received orally 100 grams of fructose, 50 grams of xylose, 50-100 grams of casein and 45-80 gm of fat (lipomal). Also studied was the effect of insulin with and without glucose, oral glucose plus KCl to prevent serum potassium from falling and oral glucose following periods of fasting up to 36 hours. Conflicting reports are present in the literature as to whether or not ADH alters urinary divalent cation excretion out of proportion to changes in urinary sodium. A maximum water diuresis was established in 4 subjects followed by infusion of ADH at 25 mμ/hr. and finally reestablishment of a maximum diuresis. Matched control studies were run on the same subjects.

High urinary sodiums were found in some subjects following an overnight fast and oral water load which could promptly be returned to normal levels following ingestion or infusion of glucose. Twenty-two normal subjects, age 19-93, following control periods, received 100 grams of glucose orally. Urines and bloods then were collected for 80 to 140 minutes following this ingestion. In selected subjects, studies were repeated using intravenous glucose with or without insulin.

All patients with hyponatremia (serum sodiums less than 125 mEq/L which have been admitted to the wards of Baltimore City Hospitals over the last 1 1/2 years have been evaluated clinically. Sixteen patients have been studied in detail with serum and urine electrolyte, renal function studies, blood volume determination (I^{131} albumin), red cell electrolytes, responses to salt or water loads and determination of steroid levels (aldosterone, cortisone). From this group, 4 patients were found to have unique problems with abnormalities in the excretion of water and salt leading to hyponatremia. Workup, including stays on a metabolic ward, resulted in documentation of syndromes of inappropriate ADH release with water retention in patients with hypothyroidism, head injury (2) and parahypopituitarism with selective glucocorticoid deficiency.

Particular attention was paid to the possibility that some acutely and chronically ill patients (advanced tuberculosis, malignancies, febrile illnesses, etc.) might be developing hyponatremia because of inability to normally conserve sodium after limited periods of fasting. To date, sixteen acutely and chronically ill patients without significant hyponatremia have been studied. Following control periods, infusions of intravenous glucose and insulin were given to determine the effect on urinary sodium excretions.

Major Findings: Ingestion of 50-100 grams of various carbohydrates (glucose, galactose and fructose), protein (casein) and ethonal led to two to four-fold increases in urinary calciums and magnesiums, 50% decreases in urinary potassiums and essentially no change in urine sodiums. Glucose administered

intravenously produced similar changes. Ingestion of equimolar amounts of fat or xylose failed to produce similar changes. Infusions of insulin also produced these changes. The increases in divalent cation excretion and decreases in potassium excretion could not be explained by changes in filtered load (GFR or serum calciums or magnesium levels), urine pH, urine flow, urinary solute or carbohydrate (osmotic diuresis), sodium excretion, urinary organic or inorganic anion excretions or hormonal alterations (parathyroid, corticoids, aldosterone).

Each of the nutrients observed to produce these changes is capable of increasing glucose concentrations in the renal medullary circulation. Blood glucose and insulin levels determine glucose uptake and the rate of glycolysis in the medullary tissues which derive their energy solely from glycolysis.

An increase in calcium and magnesium reabsorption and potassium secretion in the collecting ducts would best explain the observed changes. The increase in calcium and magnesium excretion and decrease in potassium excretion following nutrient ingestion may be a consequence of an increase in glucose uptake and glycolysis in the tissues.

Twenty-two normal subjects, age 17 to 93 years, were fasted overnight and given 20 cc water per Kg body weight. Some of these subjects, especially older subjects lost large quantities of sodium in the urine and this could be reversed by ingestion of 100 grams of glucose or, in some instances, infusions of glucose with or without insulin. Sixteen subjects with acute and chronic illnesses (tuberculosis, malignancies, etc.) were similarly studied substituting intravenous glucose and insulin for oral glucose. No evidence was found to suggest that these subjects lost large amounts of sodium in the urine after an overnight fast and oral water load.

We can conclude from these studies that, although some normal persons fasted overnight and water-loaded lose inappropriately large amounts of sodium, this doesn't appear to be the cause of the hyponatremia seen in many acutely and chronically ill persons. This inappropriate sodium loss can be corrected by ingestion or infusion of glucose suggesting that an absence of optimal amounts of glucose (utilized for energy production) leads to an inability to reabsorb or conserve sodium optimally.

Proposed Course of Project: An attempt is being made to determine why some older subjects after an overnight fast and water load lose large quantities of sodium in the urine and why this can be reversed with oral or intravenous glucose with or without supplemental insulin.

Manuscripts are being prepared describing the influence of various nutrients on urinary divalent cation excretion. Two case reports are being prepared describing unique problems in hyponatremic subjects. Workups include data collected on the metabolic balance ward.

Serial No. HD-AG19(c)
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on the effect of age on the renal handling of acid and base loads

Previous Serial Number: NHI - 192 (c)

Principal Investigators: S. Adler (1/3 time)
R. D. Lindeman (1/4 time)
M. J. Yiengst (1/4 time)

Other Investigator: N. W. Shock

Cooperating Unit: Baltimore City Hospitals

Man Years:

Total:	1.33
Professional:	.83
Other:	.5

Project Description:

Objectives: Older subjects are unable to correct an induced acidosis as rapidly as young subjects. An attempt has been made to determine if this is due to a defect in the ability to excrete titratable acid, ammonia, or both in the urine.

Methods Employed: Acute oral acid loads, in the form of ammonium chloride gelatin capsules (0.1 gm. per Kg. body weight) have been given to adult male volunteers. Twenty six subjects (ten in group I, 17 to 35 years; seven in group II, 48 to 65 years; nine in group III, 72 to 93 years) first received 10 cc of water per Kg. of body weight to establish a good urine flow. Urines were then collected at one hour intervals. After two control periods, the acid was administered and urines and blood were collected for 8 additional one-hour periods. Bloods were collected prior to NH_4Cl administration and 2, 3, 5 and 7 hours after the acid load. The levels of total CO_2, titratable acid, ammonia, sodium, phosphate and creatinine were determined on each urine. Serum pH, pCO_2, total CO_2, ammonia, sodium and creatinine were determined on each blood sample. Inulin and PAH clearances were determined during the course of each study. In an attempt to determine if the rate of ammonia production in the kidneys of older persons is limited by the amount of enzyme, glutaminase, available in the kidney, four young and four old subjects were restudied and given the NH_3 precursor glutamine

(0.25 Gm. per Kg. body weight) at the beginning of the 4th and 5th hours.

Major Findings: In all age categories, blood pH, pCO_2 and total CO_2 before acid loading were similar. The acid load caused equal falls in total CO_2 in all age groups. The younger subjects by the end of eight hours had restored their bicarbonate levels closer to normal than the older subjects. Urine pH values were lowered more in the older subjects than they were in the young. Both titratable acid and ammonia excretion decreased with age at a rate parallel to the decrease with age in glomerular filtration rate. The titratable acidity factored by GFR was equal in the 3 age groups; the ammonia factored by GFR was slightly decreased in the older age groups. To determine if this relative decrease in ammonia excretion was a result of a limitation in the enzyme responsible for ammonia formation in the kidney, glutaminase, or a decrease in the major ammonia-forming substrate glutamine, glutamine was given orally after the acid load had been administered. Serum glutamine levels prior to glutamine ingestion were identical in the two groups. The percent increase in ammonia excretion was equal in these two groups, thus excluding the above two considerations as explanations for the relative decrease in ammonia excretion with age.

Creatinine clearances have been followed on each longitudinal subject at 1 1/2 year intervals. To date, 382 longitudinal subjects have had two 24-hour clearances and 192 of these subjects have had their third 24-hour clearances. These data are now in the hands of the statistician prior to preparation of a manuscript describing the preliminary results with the use of this test.

Proposed Course of Project: This study has been completed and a manuscript is being prepared covering these findings. The creatinine clearances will be followed in longitudinal subjects at regular intervals. A manuscript is being prepared on the preliminary results.

Serial No. HD-AG20
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Intramitochondrial pH: The reaction of mitochondria to changes in external acidity and electrolyte content.

Previous Serial Number: NHI - 193

Principal Investigators: S. Adler (1/3 time)
M. J. Yiengst (1/4 time)
R. D. Lindeman

Other Investigatore: None

Cooperating Unit: Baltimore City Hospitals

Man Years:
Total: .47
Professional: .47
Other: none

Project Description:

Objectives: An attempt will be made to determine the pH of viable mitochondria in vitro. Some idea can be obtained of the pH conditions under which mitochondrial enzymes function and their responsiveness to changes in acidity. Further studies will allow us to determine if there are any tissue or age differences in mitochondrial pH.

Methods Employed: Mitochondria were isolated from rat liver using standard technique of differential centrifugation. They were then tested for intactness by measuring respiratory control with the oxygen electrode and a specially constructed cuvette. The mitochondria were then incubated in a water jacketed chamber at 25° for 10 and 20 minute periods with the pH of the medium being measured by a pH sensitive glass electrode. Samples removed at these periods were analyzed for potassium content using flame photometry, water content by overnight drying in a 50° vacuum oven, and pH by counting the C^{14} DMO in a triCarb liquid scintillator. Extra mitochondrial water was measured using PVP which was analyzed by an iodometric titration.

The incubation medium has been a 30 mM Tris Chloride buffer containing PVP, CMO^{14}, 20 mM KCl, 1mM ATP and sufficient sucrose to make a final osmolarity of 300 m Osmolar.

Major Findings: The water content and PVP spaces of the mitochondria have been determined and are fairly constant from experiment to experiment with excellent reproducibility within each run. Mitochondria have had excellent respiratory control before and after the incubation period.

The calculated intramitochondrial pH was found to be constant from 10 to 40 minutes. The pH was alkaline as compared to medium pH and remained so when pH of the medium was varied between 6.90 and 7.60. Mitochondrial pH, when medium pH was 7.10, was 7.38. The curve described by mitochondrial pH as a function of medium pH is a straight line.

Potassium content of the mitochondria demonstrated tightly coupled particles since the concentration of K in intramitochondrial water is approximately 2 1/2 times that of extramitochondrial. Furthermore, there appears to be a tendency for (K^+) to rise as pH of the medium is increased.

Proposed Course of Project: This project will be terminated July 1, 1966 because of termination of the tour of duty of the principal investigator.

Serial No. HD-AG21
1. Gerontology Branch
2. Endocrinology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The renin-angiotensin system. Chemical-radioisotope derivative assay of plasma renin and angiotensin.

Previous Serial Number: NHI-163

Principal Investigator: Robert I. Gregerman (70% time)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years:

Total:	1.7
Professional:	0.7
Other:	1.0

Project Description:

Objectives: A major obstacle to progress in the field of the renin-angiotensin-aldosterone system has been lack of any physico-chemical method for the estimation of plasma renin and/or angiotensin. All work to date has required bioassay methods. It is the purpose of this work to develop a method for the determination of the enzyme renin in which the product of enzymatic activity, angiotensin, is measured. Recent work elsewhere has re-emphasized the urgent need for such a method, since plasma renin determination appears to be indispensable for the diagnosis of primary aldosteronism, a surgically remediable disease which may be present in as much as ten or twenty percent of the hypertensive population. In addition, development of an extremely sensitive method for the measurement of angiotensin would probably serve as a model for the development of methods of measuring other small peptides of biological interest.

Methods Employed: Our general approach is one similar in principle to the double-isotope derivative assays used by workers in the steroid field. The unknown but sub-microgram quantity of material is reacted with a radioactive reagent of high specific activity, thereby converting it to a radioactive derivative. Carrier derivative, separately prepared with another isotope, is added and the combined materials purified by chromatography. The radioactivity is counted. One can then calculate the quantity of the unknown corrected for losses by the carrier's second isotope. Peptides heretofore

have not been measured by any such approach. The project concerns itself with the development of a radioisotope derivative assay for peptides and specifically for angiotensin and related peptides.

Major Findings: In our last report we described the selection of a radioactive reagent, 2, 4-dinitrofluorobenzene labeled with tritium (DNFB-H^3) and the chemical and chromatographic characteristics of the derivative formed by its reaction with angiotensin. The double label approach was described in detail. Since that time progress toward a workable assay sensitive in the nanogram range has been considerable. A new chromatographic system utilizing thin layers of silica gel on plastic has been employed. Electrophoresis of the DNP derivative on thin layers and in acrylamide gel has been explored. Conversion of dinitrophenylated angiotensin (DNP angiotensin) to its monomethyl and dimethyl esters and their chromatographing has been studied. Finally, the quantitative conversion of nanogram quantities of angiotensin to its corresponding heptapeptide by carboxypeptidase and subsequent conversion to and chromatography of the resulting DNP-heptapeptide and ester derivatives has been studied. All of these manipulations of DNP-peptides will serve as the basis of future assay procedures.

The partial measurement of angiotensin (octapeptide) over the range of 2 to 40 nanograms (1 nanogram = 1 mμg = 10^{-9} grams) has been accomplished using the following scheme. The peptide is reacted with DNFB-H^3 for 18 hours. A tracer amount of C^{14} labeled DNP angiotensin is added to serve as a monitor of the recovery of H^3 labeled angiotensin formed by reaction with DNFB-H^3. The mixture is acidified and extracted six times with unlabeled DNP angiotensin carrier added and pre-extracted with ethyl acetate. The ethyl acetate extract is dried over sodium sulfate, evaporated to dryness under nitrogen, and redissolved in a small volume of acetic acid - acetone for chromatography on a thin layer of silica gel. Following two dimensional chromatography (butanol-pyridine-water vs. methylethylketone-water-acetic acid) the yellow DNP angiotensin area is eluted from the silica gel, evaporated to dryness, redissolved and counted for C^{14} and H^3 in the liquid scintillation counter. Recoveries of C^{14} range from 20 to 40 percent overall. The yields of H^3 labeled product are close to theoretical (based on the specific activity of the DNFB-H^3 used. While not enough determinations have been prepared to permit precise estimation of the accuracy, the error at very low levels (2 nanograms) may be as high as 25% while at higher levels (10 nanograms or more) the error should not exceed 10%. Nanogram amounts of angiotensin have been assayed with similar results following enzymatic conversion to heptapeptide by the action of carboxypeptidase and isolation of DNP-heptapeptide using the corresponding stable and C^{14} labeled heptapeptide as carrier and indicator respectively.

Proposed Course of Project: In order to apply the double isotope technique to plasma renin determination it will be necessary to standardize the assay in terms of angiotensin decapeptide or its heptapeptide derivative. This is necessary since the initial product of renin action on plasma substrate is angiotensin I (decapeptide). In the proposed assay decapeptide will be

adsorbed to an ion exchange resin added to serum in which the renin is to be assayed. The resin will be washed and the decapeptide eluted. For assay of the heptapeptide the decapeptide will be converted to heptapeptide with carboxypeptidase. Double isotope assay will then be performed using the approach described. Further work will also be done on conversion of the DNP heptapeptide to its ester and rechromotography of the ester prior to counting, since preliminary experiments suggest that considerable reduction in blank and increase in overall sensitivity should result. The alternative to the heptapeptide approach is the direct measurement of decapeptide. This should be possible in exactly the manner described for either the octapeptide or heptapeptide. The only barrier to a direct approach is the unavailability of angiotensin I. We are currently engaged in an attempt to synthesize this material and appear to have succeeded in obtaining the protected decapeptide, CBZ-aspara-arg-val-tyr-val-his-pro-phen-his-len from the corresponding protected octapeptide and his-ten methyl estes by the carbodiimide route. The product(estimated 75% purity) requires purification and hydrogenation, upon completion of which one should obtain a desired compound. In the unlikely event that the final material fails to be satisfactory, it will be necessary to accomplish a total synthesis utilizing the solid state method. In the meantime, however, the heptapeptide-carboxypeptidase technique seems to be a wishable alternative. As soon as these matters are settled, application of the assay to the determination of plasma renin will be undertaken.

Serial No. HD-AG22
1. Gerontology Branch
2. Endocrinology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Relationship of age to Thyroid function and Thyroid hormone metabolism.

Previous Serial Number: NHI-161

Principal Investigators: Robert I. Gregerman (20% time)
J. Howard Lutz (100% time)

Other Investigators: Albert Dawkine
Richard Hornick

Cooperating Units: Baltimore City Hospitals
University of Maryland School of Medicine

Man Years

Total	2.20
Professional	1.20
Other	1.00

Project Description:

Objectives: This study is a continuing effort to learn more about the effects of age on thyroid function and hormone metabolism. Previous investigations from this laboratory have concerned themselves with the relationship of age to Thyroidal and renal radioiodide handling, plasma hormone concentrations, and the peripheral turnover of thyroxine in man and animals. Previous work has also shown that during acute bacterial pulmonary infections in man the rate of thyroxine degradation is markedly accelerated. This finding has now led us to explore the current hypothesis (Robbins and Roll) that the rate of Thyroxine turnover (degradation) is determined by the plasma concentration of "free" (unbound) Thyroxine. Recent studies elsewhere have shown that during acute illness a rapid decrease occurs in plasma Thyroxine binding pre-albumin (TBPA); in consequence, there is an increase in plasma free Thyroxine. We are attempting, therefore, to correlate changes in TBPA, free Thyroxine, and thyroxine turnover kinetics during febrile illness. At the same time, since our previous studies have shown that Thyroxine turnover is slowed in normal old subjects, a study of the relationships of TBPA, free thyroxine, and age has been undertaken.

Methods Employed: TBPA is being measured by the recently described saturation (binding capacity) technique of Oppenheimer in which paper electrophoresis and ^{131}I-thyroxine are used. TBPA radioactivity is located by radioautography and counted in a scintillation well detector. Free Thyroxine is being measured by equilibrium dialysis using diluted serum (Oppenheimer) and magnesium precipitation of dialysate thyroxine (Sterling). Thyroxine turnover kinetics are studied using thyroxine ^{125}I. Subjects with febrile illnesses are prison volunteers at the Maryland State Penitentiary being studied by the Infectious Disease Group of the Department of Medicine, University of Maryland.

Major Findings: To date most of our effort has gone into standardization of the methodology for measuring TBPA and free Thyroxine. Attempts to determine TBPA by acrylamide gel electrophoresis were abandoned when technical difficulties were encountered which rendered difficult densitometric quantitation of the TBPA. Instead, TBPA is being measured by the saturation technique.

A series of five subjects have had thyroxine turnover determined before, during, and after injection with the organism of tularemia (Pasteurella Tularemia). The subjects, though medically ill and severly febrile for periods up to a week, showed no alteration of thyroxine turnover from normal. Determinations of free thyroxine and TBPA are in progress. The lack of acceleration of Thyroxine turnover is in sharp contrast to our previous results with bacterial pneumonias, but similar to the finding of Wiswell and Coronko in typhoid fever. If, as expected, TBPA decreases and free T_4 increases during the febrile phase, the evidence will be very strong that free T_4 is not a determinant of thyroxine turnover and will serve to answer definitively the question under consideration. On the other hand, further studies in other infectious illnesses which are accompanied by acceleration of known illnesses may be necessary should tularemic subjects fail to exhibit TBPA and free T_4 alterations. In any case, it is already clear from the study with tularemia that neither fever nor hypermetabolism, per se, are determinants of thyroxine turnover.

Proposed Course of Study: A number of other infectious diseases will be studied (Malaria, Q-fever, etc.), since the nature of the bacterial disease appears to be a significant factor in the response seen. Systematic study of the binding proteins (TBPA and thyroxine binding globulin) and of free T_4 concentrations in normal subjects as they are related to age are being undertaken.

Honors and awards:

Dr. Robert I. Gregerman was elected a fellow of the Clinical and Biology Section of the Gerontological Society at the Society's 1965 meeting in Los Angeles, California.

Serial No. HD-AG23
1. Gerontology Branch
2. Endocrinology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Influence of age on aldosterone secretion: Aldosterone secretory rate determinations and metabolic balance studies on normal subjects.

Previous Serial No. NHI-162

Principal Investigators: Robert I. Gregerman (5% time)
C. Robert Cooke

Other Investigators: None

Cooperating Units: Baltimore City Hospitals, Department of Medicine
Johns Hopkins University School of Medicine, Department of Medicine

Man Years:

Total:	0.05
Professional:	0.05
Other:	none

Project Description:

Objectives: This project is designed to study quantitatively the effect of age on the adrenal gland's secretion of aldosterone in normal human subjects. A variety of maneuvers known to affect mineralo-corticoid secretion will be employed. The data obtained will establish normal standards for evaluating the role of aldosterone in the elderly hypertensive subject.

Methods Employed: Aldosterone secretory rate determinations and metabolic balance studies have been performed on eight normal subjects whose ages have ranged from 25 to 67 years. Test subjects were admitted to the Osler V Clinical Research Unit of the Johns Hopkins Hospital where balance data were collected during two consecutive study periods of 5 - 7 days duration each. A constant diet containing 10 mEq Na^+ and 60 mEq K^+ was taken during both periods. Body weight and B.P. were recorded twice daily and all urine and stool were collected for determinations of Na^+, K^+, Cl and total nitrogen excretion. Sodium intake was increased to 180 mEq / day during the first period by NaCl supplementation and was restricted to the amount provided by the constant diet during the second period.

At the end of each period, H^3-d-aldosterone (1-3μC) was injected intravenously and urine was collected during the subsequent 24 hours for determination of aldosterone secretory rates by a modification of the method of Kliman and Peterson. Samples of blood were also obtained at these times for determinations of plasma renin activity by bioassay. Inulin and PAH clearance studies were performed on the day before or the day after the performance of aldosterone secretory rate determination. Creatinine clearances (24 hours) were also determined periodically during each of the two study periods.

Major Findings: Aldosterone secretory rate values determined thus far have ranged from 34 micrograms/24 hours to 159 micrograms/24 hours during the period of augmented sodium intake, and from 76 micrograms/24 hours to 253 micrograms/24 hours during the period of sodium restriction. The number of patients studied is too small as yet to determine the correlation between aldosterone secretion and age of the subject studied, but the investigation will continue until sufficient data are available.

Serial No. HD-AG24
1. Gerontology Branch
2. Endocrinology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Work performance of rat heart-lung and isolated heart preparation: standardization and influence of aging.

Previous Serial No: NHI-165

Principal Investigator: N. Solomon (50% time)

Other Investigators: N. W. Shock
A. Pollack

Cooperating Units: Baltimore City Hospitals
Department of Physiology, University of Maryland Medical School

Man Years
Total: 1.50
Professional: .50
Other: 1.00

Project Description:

Objectives: To explore the mechanisms responsible for the reduced work capacity of the senescent rat heart.

Methods Employed: Both the isolated rat heart-lung preparation (described in last report) and the isolated rat heart preparation were used to assess the functional capacity of the myocardium, expressed as LVWI (left ventricular work index) in g-Meters per 100 mgm. dry heart weight. The isolated rat heart was chosen in order to clearly delineate the effects of aging on myocardial function. In this preparation the lungs were excluded from the circuit. The circuit for the outflow of blood from the heart was exactly the same as that described for the rat heart-lung (via the cannulated right common carotid artery). The circuit for the inflow was not through the inferior vena cava, but through a cannulated pulmonary vein. The inferior vena cava was ligated. Coronary blood flow was obtained by measuring the flow through the cannulated pulmonary artery.

Major Findings: The number of heart-lung and isolated heart preparations studied for each group was increased from 13 to 18, and from 5 to 15 respectively. The data unequivocally confirm that the heart of the senescent rat demonstrates a significant impairment in myocardial work capacity. Left ventricular work index (LVWI), expressed in gram-meters per 100 mgm. dry heart weight for the heart-lung preparation, was 34 ± 4 (24 mo.) vs. 166 ± 14 (12 mo.) ($p < 0.01$). LVWI for the isolated rat heart was 15 ± 5 (24 mo.) vs. 77 ± 13 (12 mo.).

Because of the extreme surgical difficulty in isolating the rat heart preparation, as demonstrated by the low survival ratio of one per four animals attempted, and because the data from the much more surgically feasible heart-lung preparation were qualitatively similar, the heart-lung preparation will be used subsequently as the standard preparation whenever the data sought make it possible.

Effects of temperature of perfusing blood on LVWI of isolated heart:
At a perfusion blood temperature of 37° C heart rate was higher and LVWI lower than at 32° C. The number of experiments has been increased from 5 to 15 twelve month male animals in each group. MABP was 130 mm Hg., and LVWI was 159 ± 15 (32° C) vs. 77 ± 13 (37° C).

Effect of changing peripheral resistance on LVWI of isolated heart:
Peripheral resistance was adjusted to give an analysis of work performance at three different levels of mean arterial blood pressure: 80, 110, and 130 mm Hg. The lower the pressure, the greater the LVWI. However, the heart rate was not influenced by alterations in peripheral resistance. Since the last report the number of experiments has been extended from 5 to 15 twelve month old rats in each group and LVWI was 163 ± 19 (80 mmHg.), 122 ± 20 (110 mm. Hg.), and 77± 13 (130 mm. Hg.).

Substantiation of work performance with the effects of epidemic myocarditis:
Because of the possibility that the poor work performance of the three month hearts was a result of the epidemic myocarditis found upon pathological examination, a new series of experiments was performed on three month hearts which were subsequently proven free of myocarditis. The LVWI's from groups of eighteen 3, 12, and 24 month rats were 160 ± 16, 166 ± 14, and 31 ± 4 respectively. The data clearly demonstrate that there is no significant difference in work performance between 3 and 12 month animals. However, the 24 month rat shows a profound decrease in work capacity ($p < 0.01$).

Mechanisms of failure:
Pilot studies were undertaken to determine both the mechanisms of failure involved and the mechanism by which the senescent heart fails more rapidly.

The pH of the prefusing blood was measured before the experiment was run and again after the heart had failed. The data show that there is no significant difference in the pH value, and in nine experiments, the pH was 7.4 ± 0.03 at the beginning of the experiment and 7.39 ± 0.03 at the termination.

The possibility that the S-A node might fail, thereby causing the failure of the experimental heart was next considered. A stainless steel micro-electrode was implanted in the region of the sino-auricular node, and a standard heart-lung preparation run. After the heart could no longer maintain a pressure of 110 mm Hg., the heart was stimulated electrically by a 5-S Grass Stimulator at a rate of 360 stimulae per minute for a duration of .6 milliseconds at 2 volts. The heart did not respond with a rate increase, and the EKG registered fibrillation. The fact that electrical stimulation proved unsuccessful in

reviving the failed heart leads us to believe that a biochemical change in the perfusing blood and /or in the heart itself is responsible for causing failure.

Several studies were conducted on the blood of 12 and 24 month animals to determine if a change in a biochemical component of the blood of senescent rats could cause a premature failure.

The titre of corticosterone was measured in 3, 12, and 24 month male rats and the following data obtained in micrograms per hundred ml. plasma: 37 ± 5, 29 ± 7, and 33± 8 respectively. There was no significant difference in the level of circulating corticosterone as a function of age.

The following blood studies were performed on 12 and 24 month male rats: Na, K, Cl, CO_2, P, Ca, Osm., Alk Ptase, Uric Acid, Urea N, Creat, T.P., A/G, Ceph Floc. Thymol S-H units, Bilirubin, SGOT, SGPT, LDH, Chol, T^4, Glucose, and Electrophoresis.

There was no significant difference in the values obtained on 12 and 24 month animals with the exception of circulating thyroid hormone. In 12 twelve month males, the thyroxine-bi-column was 2.4 ± .2, and in six 24 month males the thyroxine-bi-column was 0.5 ± .15 ($p < 0.01$). These low values warrant further investigation.

Proposed Course of Project: These studies will continue to probe the mechanisms of failure. The biochemical studies will include an analysis of myocardial substrate utilization and energy production and utilization as a function of age. Comparative electrolyte and steroid determinations will be performed on the perfusate before and after failure.

Myocardial Na and K flux ratio analysis will be performed as a function of age.

Twelve month rats will be perfused with senescent blood.

Thyroid hormone will be added to the senescent heart preparation.

Studies with electrical stimulation will be continued. Hearts of various ages will be stimulated at predetermined rates and the influence of this variable on the functional capacity of the senescent rat heart determined.

Publications

1. Solomon, N., Carpenter, C. C. J., Bennett, I. L., and Harvey, A. McG.: Schmidt's syndrome (thyroid and adrenal insufficiency) and coexistent diabetes mellitus. Diabetes, 14: (5), 300-304, May 1965.

2. Wagner, H. N., Migita, T. Jr., and Solomon, N.: Effect of age on reticuloendothial function in man. J. Geront., 21: (1), 57-62, Jan. 1966.

Serial No. HD-AG25
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Operant responding of rats, hamsters, and mice as a function of age

Previous Serial Number: NHI - 203

Principal Investigator: C. L. Goodrick (20% time)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years
Total: .40
Professional: .20
Other: .20

Project Description:

Objectives: To determine changes in operant performance, discrimination learning and extinction as a function of age.

Methods Employed: Animals are run in standard operant conditioning Skinner Boxes. Bars which activate relays, timers and feeders are standardized for animals of differing species or strain. Studies are concerned with differences in operant level, light contingent bar pressing, operant responding for food or water rewards, extinction while nondeprived, food and/or water deprived and the relations of measures obtained to species or strain and age. Typically experiments are designed to yield the maximum information for a minimum number of animals. Animals are usually placed in 2-bar Skinner Boxes so that, e.g., light contingent bar pressing may be determined directly by comparisons of bar preferences, rather than through the use of control groups.

Major Findings: 1. Control experiments have been concerned with the nature of light in relation to primary and secondary reward. In earlier experiments a stimulus (light) formerly associated with primary reward was made contingent on a press of a nonreward bar after a series of retention trials. After

the initial trial, young rats went back to press the bar formerly associated with primary reward, although older rats failed to do so. The present experiments have shown that under these conditions light functions as a primary reward rather than a secondary reward. Four groups of food deprived young male rats were run for milk-sucrose reward in a 2-bar Skinner box on continuous reward. Two groups were trained with a light correlated with the primary reward and two groups in the dark. During retention tests one of each pair of groups was extinguished in the dark and one pair with a light contingent upon pressing the reward bar. After a series of trials, the light was made contingent upon a nonreward alternate bar. Light contingent bar pressing did not increase rate of pressing for either group in comparison with rats in the 'dark' boxes. However both groups of rats initially tested in the dark increased response rates on the nonreward bar when the bar press was made light contingent. This clearly shows that the shift was due to the function of light as a primary reward rather than as a secondary reward.

Early studies within this section have shown (a) that the primary reward value of light is similar for young and senescent rats and (b) senescent rats tend to extinguish a preference to a reward bar upon switching bars on initial light contingent tests during extinction in comparison with young rats which do not switch. Finding (b) is therefore shown to be related to behavioral rigidity in the senescent rats, rather than a secondary reward effect.

Proposed Course of Project: Experiments have been designed to determine age differences in: 1. Extinction as a function of spaced versus massed extinction trials over extended periods. 2. Rate of responding and extinction as a function of quantity and quality of reward. 3. Maintenance of food and water ingestion over extended periods by bar pressing within the home cage. 4. Performance and extinction as a function of effortfulness of the response. 5. Alternation behavior between two bars as a function of probability of reward. 6. Bar pressing for light onset and offset as a function of light intensity.
In addition, preliminary experiments are being devised to obtain data for Golden Hamsters and various inbred strains of mice and examine the role of secondary reward schedule during extinction upon the retention of the bar pressing habit.

Honors and Awards: None

Publications:

Serial No. HD-AG26
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Fluid preferences of rodents as a function of age

Previous Serial Number: None

Principal Investigator: Charles L. Goodrick (40% of time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years
Total: .80
Professional: .40
Other: .40

Project Description:

Objectives: To determine fluid preferences and absolute and differential gustatory thresholds as a function of age, strain and species.

Methods Employed: Animals are run in their home cages. Calibrated drinking tubes are utilized to precisely measure fluid intake. For measurement of the absolute preference threshold two bottles are within the cage. One bottle contains water (standard) while the other, the comparison, contains a given percentage of the substance for which the threshold is being obtained. Bottles are switched randomly so that response variability is controlled as well as possible. For differential preference thresholds a fixed standard solution (e.g., 1 gm sucrose/100 ml.) is compared with a series of alternate comparison solutions (e.g. 3,2,1.5 gms. Sucrose/100 ml.). Other methods may involve single or multiple bottle tests, water deprivation and ingestion during or over specific time intervals.

Major Findings: 1. Quinine and sucrose discrimination was examined for 1, 5, 15 and 25 mo. old male rats (N=80), utilizing 48-hour 2-bottle tests with serially decreasing concentrations of comparison solution. Standard solution was water. Older rats had less ability to discriminate the aversive stimulus of quinine and the preferred stimulus of sucrose than young rats. Tests for the alternate substance were given after quinine and sucrose tests to determine possible interactions of past experience with age. Past experience with sucrose increased quinine ingestion, while experience

with quinine decreased sucrose ingestion, as compared with controls. For old animals, these effects required higher comparison concentrations than for young animals.

2. The differential threshold (DL) for sucrose was studied with 2-bottle tests in male rats 1, 5, 15 and 25 months old (N= 11/group). Standard sucrose solutions were 1, 5 and 9 grams/100 ml., with comparison values of the following multiplicative values: 3, 2, 1.5, 1.25 and 1.125. In general, for standard solution of 1 and 5 grams the DL was an increasing function of age. However, with a standard of 9 gms. 5./100 ml. the preference for a comparison solution of 27 grams was directly related to increasing age, with 1 mo. old animals preferring the 9 gram standard solution and 25 mo. old rats preferring the 27 gram comparison solution. Water ingestion in preliminary tests showed a directly increasing function with increasing age. However, during tests with pairs of sucrose solutions, younger rats ingested greater quantities of fluid than older rats. In addition, for young rats ingestion was greater as the standard and comparison solutions became more difficult to discriminate. This increase was not observed for senescent rats. Although this experiment clearly shows a decrement in discrimination for senescent rats, other factors such as differential age effects at low and high sucrose concentrations must be considered in age-threshold comparisons. Also, younger rats clearly show a greater ability to change fluid volume intake than senescent rats when presented with highly palatable solutions.

Proposed Course of Project: Based on the data of experiment 2 several hypotheses may be tested: 1. It is suggested that the increases in volume intake for young rats when comparison and standard solutions are very similar (in comparison with no increase for senescent rats) may be due to increased shifting from comparison and standard to sample the stimuli. This hypothesis may be tested by recording directly ingestion from both bottles. Contact switches have been constructed for this purpose.

2. The shift to the standard 9 gram bottle by 1 month old rats may be due to water requirement in the "fast growth" stage. This hypothesis may be tested by allowing a third bottle (water) during testing. The hypothesis is that growing rats will ingest more water than older rats and the relative preference would shift to the comparison bottle with the high sucrose concentration (27 grams/100 ml.).

Serial No. HD-AG27
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: An experimental analysis of rodent exploratory behavior as a function of age.

Previous Serial Number: NHI-204

Principal Investigator: Charles L. Goodrick (40% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years
Total: .80
Professional: .40
Other: .40

Project Description:

Objectives: 1. The development of reliable and valid tests which measure exploration; 2. The determination of the amount of exploratory behavior under specified conditions as a function of age and heredity; 3. The specification of variables which control such behavior.

Methods Employed: Time sample methodology has been utilized in all experiments. Such research involves taking measures systematically during a particular time interval. In the present work several different test areas and time intervals have been used.

To determine exploration of rodents, an environment has been developed which allows measurement of normal behavior, i.e., sniffing, grooming, or lying, as well as preference for light or comparative darkness. By placing an animal within the environment for a fixed period of time and sampling behavioral and place responses, a valid measure of exploration may be obtained. Such tests have been used to determine responses of rats and mice. For other research, variations of the above test which are more or less complex will be devised.

Major Findings: Male albino rats are being run on a variety of tests to determine interrelations of various measures of exploration and emotionality. Standard tests of exploration, previously utilized, are being related to historically earlier tests of open field ambulatory activity and "emotional" elimination. In addition, these tests will be related to wheel activity

level, rate of pressing a bar in a Skinner Box (operant level), and light or dark contingent pressing of the bar. Emotionality measures have been obtained during all tests (i.e., defecation rate). Earlier data have shown that an increase in motivation by means of food deprivation reduces differences in exploration between young and senescent rats, so that rats of various ages have been run which include groups of nondeprived and deprived rats. At present two age groups (mature-old, 15 mo.; senescent, 25 mo.) have been run. Young-mature (5 mo.) and selected groups of recently weaned (1 mo.) rats will be run to complete the behavioral age functions. This research will add substantially to the literature presently available concerning emotional characteristics of rats over the entire age range.

Although analyses are incomplete, several empirical and theoretical general findings may be tentatively summarized:

1. Senescent rats (25 mo. old) are more emotional than mature-old rats (15 mo. old).
2. Food-deprived animals of both age groups, considering all tests, may be classified as engaging in more exploratory behavior and being less emotional than nondeprived animals.
3. If light-contingent bar pressing is related to exploration, then food deprived animals should turn on a light in a dark chamber more often than nondeprived animals; however, nondeprived animals within a lighted chamber should turn a light off proportionally more than food deprived animals. This theoretical prediction has been verified for both age groups.

Proposed Course of Project: The project discussed will be completed and data analyzed. Experiments are planned which will allow an evaluation of aging upon drive activation and fighting behavior. In other experiments, drugs will be utilized to change level of activity and exploration. Emphasis will also be upon environmental methods of increasing the exploratory behavior of senescent rats.

Serial No. HD-AG28
1. Gerontology Branch
2. Molecular Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Structure of nucleic acids

Previous Serial No.: NHI - 172

Principal Investigators: G. L. Eichhorn (75% time)
Patricia Clark (60% time)
Yong Ae Shin (100% time)
J. Sri Ram (10% time)

Other Investigator: None

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	3.8
Professional:	2.4
Others:	1.4

Project Description:

Objectives: (1) To study the reactions of metal ions, proteins and synthetic polypeptides with nucleic acids. (2) To find specific reactions of the individual nucleotides. (3) To understand the biological significance of metal ions in nucleic acid function. (4) To develop methods for the determination of nucleotide sequence in nucleic acids.

Methods Employed: Conditions are determined for the binding of metal ions to (1) the phosphates on polynucleotide chains and (2) the bases attached to the ribose phosphate backbone. Metal binding to phosphate can be used to degrade the polymers, and the degradation reactions are studied for possible specificities in their attack on different internucleotide bonds. Metal binding to the bases competes with the hydrogen bonding of these bases and thus affects the helix-coil transitions of the polymers. Protein binding to the phosphates simulates a condition of high electrolyte concentration. Through studies using a combination of protein and a metal which binds to the bases it is hoped to find clues to the participation of metal ions in biological phenomena such as DNA replication and protein synthesis.

Preferential binding of metal ions to some of the bases may be used in sequential analysis either by rendering the affected bases more susceptible to hydrolytic attack or by specifically inhibiting the attack of nuclease enzymes upon the metal bound nucleotides.

Major Findings: A. Non-enzymatic degradation of polynucleotides. One of the principal goals of the studies on the interaction of metal ions with polynucleotides has been to find chemical methods for cleaving a polynucleotide chain at specific points on the chain. If these points are determined by the nature of the nucleotide base, such specific cleavage would provide a useful tool in sequence determination. We have now found that the cleavage of polyribonucleotides by zinc will occur preferentially at sites other than those occupied by guanosine. In order to arrive at this finding, we have worked out a method for determining precisely at what sites on the polyribonucleotide chain cleavage has occurred. The method consists of treating the cleaved oligonucleotides resulting from the zinc degradation reaction with phosphatase to split off the terminal phosphates, then reacting with alkali to cleave the remaining phosphodiester linkages, and finally separating the resulting nucleotides from the nucleosides and identifying the nucleosides. Relatively little guanosine nucleosides are detected after this procedure has been followed.

We have previously shown that the degradation of polyribonucleotides brought about by zinc, is inhibited by silver. This inhibition is more pronounced in the case of homopolymers of inosine and uridine. Studies are now underway to see if this zinc-silver countereffect can be employed in obtaining some specificity in the degradation products. Preliminary studies have shown that when zinc + silver were employed, the degradation of RNA took place with a specificity different from that obtained when zinc was employed alone.

B. Interaction of Cu(II) with DNA, nucleotides, and nucleosides. The renaturation of copper-denatured DNA has been found to be a second-order reaction. This finding is explained by the fact that the bases on the two separated strands produce hypochromism in each of the two chains during the renaturation. Spectrophotometric titration of denatured DNA with copper ions has indicated that 1 to 2 moles of copper react with each DNA nucleotide.

Nuclear magnetic resonance studies on deoxynucleosides and deoxynucleotides in dimethyl sulfoxide have revealed that copper does not react with the amino groups or protonated heterocyclic nitrogen atoms on any of the bases, thus pinpointing the metal attachment at N_7 for both purines and N_1 for cytosine. The inability of thymidine to react with copper is confirmed.

C. DNA-poly-L-lysine interactions and the effects of metal ions. Intensive investigation on the interaction of DNA with poly-L-lysine as a histone model, and the effects of metal ions on this interaction are now underway. The initial studies consisted of a search for methods that can be used to follow nucleic acid -protein interaction. Two that have been found useful are optical rotatory dispersion (ORD) and melting curves. Conductometric titration and infrared spectroscopy appear promising.

DNA and poly-L-lysine interaction. Examination of the optical density-temperature profile of very dilute solution in low salt concentration indicate that there are two transitions, one corresponding to the DNA and the other to DNA:poly-L-lysine complex. The ratio of the two species corresponding to the hyperchromicities of the two transitions show that the DNA:poly-L -lysine binding reaction is quantitative, and the complex has a definite stoichiometric ratio of 1:1. The second transition (DNA:poly-L-lysine complex) is insensitive to salt concentration ($NaClO_4$) below 0.1M.

Cooling studies show a direct proportionality between the amount of poly-L-lysine and the extent of optical density lowering.

Melting studies with the metal ions in DNA, DNA:poly-L-lysine complex. Both transition temperatures of the DNA and DNA:poly -L-lysine complex are markedly lowered by the presence of copper ion. There are two more intermediate transition steps, perhaps due to the presence of DNA species which are only partially covered by poly-L-lysine. It is interesting that these intermediate transitions appear clearly only in the presence of copper ion.

The presence of magnesium ion causes Tm rise in the first transition corresponding to the free DNA. However, the second transition due to the melting out of DNA:poly-L-lysine complex is not affected by the addition of magnesium ions.

Nickel and manganese ions affect both transition temperatures. The first Tm is raised, but the second Tm is lowered.

These observations indicate that transition metal ions weaken the bond between the DNA:poly-L-lysine complex, thus, decreasing the protective effects of poly-L-lysine against melting of DNA. The metal ions act perhaps as a bridge between the DNA: poly-L-lysine linkage. The order of the ability to bridge seems to be: $Cu^{++} >> Ni^{++} = Mn^{++} >> Mg^{++} = Na^{+}$

Addition of poly-L-lysine caused a decrease in the extent of renaturation of DNA brought about by sodium nitrate in the

presence of copper ion. It would appear that Cu^{++} is involved in the DNA-poly-L-lysine bridges and is not available to participate in the renaturation process.

Optical rotatory dispersion studies of the DNA and poly-L-lysine interaction and the metal ion effects are in progress. Preliminary studies indicate that there is a correspondence between the change in ORD and the amount of polylysine added.

Proposed Course of Project: The studies on the effect of metal ions in the determination of nucleic acid structure will be continued, with emphasis on reactions that provide specificity in binding to the bases or in the cleavage of internucleotide bonds. Stress will be placed on the biological implications of these metal ion reactions. In this connection studies on the effect of metal ions on the nucleic acid-histone interaction will be carried out. We have now studied metal ion-polynucleotide reactions that illustrate many of the changes that occur in nucleic acids during the replication of DNA and during protein synthesis, and believe that modifications of some of these reactions represent biochemical mechanisms.

Honors, Awards, and Publications:

Honors: 1. Gunther Eichhorn was invited to present a lecture and lead discussion at a symposium on the structure and function of DNA sponsored by the (East) German Academy of Science, Jena, July 5-9, 1966.

2. Gunther Eichhorn was invited to present a lecture at a symposium on DNA Physical Chemistry, Madison, Wisconsin, June 14-16, 1966.

3. Gunther Eichhorn was asked to write several books and to present numerous talks on recent research on the nucleic acids.

Publications:

1. Eichhorn, G. L., and Everett, W. W.: The interaction of metal ions with polynucleotides and related compounds. VI. Effect of metal ions on the anion-exchange behavior of the nucleotides. Anal. Biochem. 11: 592-594, June 1965.

2. Eichhorn, G. L., Clark, P., and Becker, E. D.: Interactions of metal ions with polynucleotides and related compounds. VII. The binding of Cu(II) to nucleosides, nucleotides, and deoxyribonucleic acids. Biochemistry 5: 245-253, Jan. 1966.

Serial No. HD-AG29
1. Gerontology Branch
2. Molecular Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Age changes in collagen

Previous Serial Number: NHI - 174

Principal Investigators: J. J. Butzow (100% time) (Left 7/31/65)
Gunther Eichhorn (10% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years
Total: .283
Professional: .183
Other: .10

Project Description:

Objectives: Objectives and methodology of the collagen project have been described in detail in last year's report. This constitutes a concluding statement on this project.

Methods Employed: Our previous work has shown that the procedure for preparing collagen from rat tail tendon, which uses a preliminary wash 0.5M sodium dihydrogen phosphate followed by swirling in a volume of 0.5M acetic acid large in comparison with the total amount of collagen, is an efficient one which can also extract material that is highly chemically crosslinked and of physically complex structure. Our previous work has also made it clear that properties of the collagen extracted from differently aged tendons must be considered in light of the amount extracted. We decided that the rates of extraction from differently aged tendons must be studied, along with the properties of the material eventually extracted and especially those of the material solubilized up to the same fraction of the total collagen.

Major Findings: The studies on collagen have been used to show under what conditions it is possible to carry out aging studies that do not require statistical methods. The changes in sedimentation and rate of solubilization with age are so drastic

that no statistics are needed. On the other hand, the viscosity and optical rotation measurements would have to be subjected to statistical analysis to determine exactly how these properties change with age.

Proposed Course of Project: The original objectives of this project have been realized and it has been discontinued. It is possible that other studies on collagen will be taken up in the future, possibly in collaboration with the Section on Biophysics.

Honor:

Gunther Eichhorn was invited to a symposium on connective tissue in Semmering, Austria, June 22-24, prior to the 7th International Congress on Gerontology.

Serial No. HD-AG30
1. Gerontology Branch
2. Molecular Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The function of metal ions in enzymatic reactions

Previous Serial Number: NHI - 173

Principal Investigators: Gunther Eichhorn (15% time)
Patricia Clark (40% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years
Total: 1.05
Professional: .55
Other: .50

Project Description:

Objectives: To arrive at an understanding of the mechanisms by which metal ions participate in enzymatic reactions, and also to determine why different metal ions are required for different enzymatic processes.

Methods Employed: It is assumed that in any metal-catalyzed enzymatic process the metal ion must serve either (1) as an active site, by direct attachment to the substrate, or (2) it must react with the enzyme protein, and thus alter the secondary and tertiary structure of the latter, producing the active configuration. It is postulated that, whenever scheme (1) applies, the metal should be capable of bringing about a non-enzymatic reaction; failure of the non-enzymatic reaction, inversely, would favor scheme (2).

Specifically selected enzymatic substrates are treated with metal ions under varying conditions, and the nature of the metal-substrate interactions detected by physical measurements, e.g., spectrophotometry. The interaction of the metals with the enzyme protein is studied by macromolecular techniques, such as ultracentrifugation.

Major Findings: It has been previously reported that Cu(II)

inhibits the hydrolysis of DNA by DNAse I, whereas most divalent metal ions, i.e., Mg(II), enhance it. To test whether this inhibition has any specificity with regard to cleavage of internucleotide bonds the smaller hydrolysis products were separated from the larger DNA fragments by precipitation with acid and the base content of the large fragments was determined. The percentage of each of the nucleotides found in these fragments is nearly the same whether these fragments are produced by a Cu(II) inhibited hydrolysis of DNA or a Mg(II) activated one. In addition, the possible effects of Cu(II) in causing specific changes in the products of DNA-DNAse interaction are being studied.

A literature search of the effect of metal ions on various RNA degrading enzymes has been carried out to provide a comparison with our findings on pancreatic ribonuclease. This search has uncovered numerous studies, many of which come to erroneous conclusions when considered apart from other work. When the studies are related to each other it is apparent that the same metals can inhibit or activate these enzymes with changing conditions of concentration and pH. Our previous findings on pancreatic RNAse can therefore be generalized for ribonucleases in general.

Proposed Course of Project: Development of new techniques for determining the specificity of metal activated enzymes is in progress. Specifically, methods for identification and estimation of bases adjacent to the enzyme susceptible bonds are being sought. In this connection new techniques will be developed for chemical modification of DNA.

Honors:

1. Gunther Eichhorn was invited as session chairman to the 9th International Congress of Coordination Chemistry, St. Moritz, Switzerland, Sept. 3-9.

2. Gunther Eichhorn organized a 4 day symposium on Biologically Significant Coordination Compounds at the American Chemical Society Meeting, Pittsburgh, March 22-31, 1966.

3. Gunther Eichhorn was elected to membership in the International Union of Pure and Applied Chemistry.

4. Gunther Eichhorn was asked to edit several books on the subject of metal ion function in biology.

5. Gunther Eichhorn was invited to present a lecture at the Alfred Werner Centennial Symposium, New York, Sept. 11-16, 1966.

Serial No. HD-AG31
1. Gerontology Branch
2. Molecular Biology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Growth, Aging and Immunological Studies

Previous Serial Number: None

Principal Investigator: J. Sri Ram (90% time)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	.52
Professional:	.52
Others:	.00

Project Description:

Objectives: It is well established that all living organisms exhibit progressive changes in metabolic activity and biochemical architecture of tissue components with passage of time. The changes in critical stages of life cycle are reflected in the kinds and quantities of intra and/or extra cellular proteins, or enzymes. This is hardly surprising since development, which is genetically controlled involves synthesis and interactions of macromolecules. Immunological techniques, due to their exquisite specificity and sensitivity provide excellent tools for establishing differences in tissue composition. Adult organ antisera have been successfully employed in demonstrating the sequential appearance of antigenic substances during embryonic development of a variety of organs of many species. So far this approach has found very limited application in gerontological research.

Karaev demonstrated that tissues from older animals contain antigens not present in those of younger animals. In these studies, Karaev examined the antigenic differences in the blood sera and saline extracts of spleen and liver of cows (3-5 years and 10 years old, respectively) by immunizing guinea pigs with tissue extracts of young or old animals. Animals immunized with the "antigens from young" were first desensitized with the heterologous "antigens from old" and vice versa. After desensitization, the guinea pigs were given shocking doses of the homologous antigen. The animals immunized with extracts of old organs, but desensitized with extracts of young organs, showed symptoms of anaphylactic shock on receiving the homologous antigen while the animals immunized with antigens from young, and desensitized with antigens from the old, showed no symptoms of shock. This suggested that extracts of old organs contained all the antigens of the young organs plus some additional ones. Similar results were obtained with tissue extracts

from white rats. The present project will be based on Karaev's report that tissues of older cows and white rats contain antigens not present in the tissues of younger animals.

In view of the importance of this report in relation to aging processes it would first be necessary to obtain independent verification of this Russian report. If the tissues exhibit age-dependent differences in antigenic composition, the following interesting questions will arise, answers to which may very well represent an understanding of the aging processes. What is the nature of these antigens? What is the mechanism of their formation? What is the role, if any, of these antigens in aging processes? Is it a product or an initiator of some biological process associated with aging? It would therefore be the objective of this project; a) to determine whether "old Tissues" contain new antigens as claimed by Karaev and b) if feasible, to characterize and localize these antigens and study their origin and activity.

Methods Employed: The tissues from the very young and very old rats will be employed as source of antigens. Both rabbits and guinea pigs will be immunized with the antigen extracts. Blood sera and saline extracts of spleen and liver, as employed by Karaev, will be studied initially.

For immunization, the "antigens" will be injected in three different media: (a) as saline solutions, (b) as alum precipitates, and (c) in complete Freund's adjuvants.

The animals will be bled at various intervals after immunization and the sera will be analyzed for the presence of specific antibodies. Antisera obtained against "old" tissue extracts will be quantitatively "absorbed" with antigens of the "young" tissues and the supernatants will be examined for the presence of residual antibodies specific for antigens of old tissues not common with young tissue extracts. In reciprocal experiments, antisera obtained against antigens of young tissues will be "absorbed" with extracts of old tissues and the supernatants will be analysed for the presence of antibodies specific for antigens characteristic of the young tissues. For these analyses, classical immunochemical techniques such as precipitin formation, immunoelectrophoresis, complement fixation, systemic and passive cutaneous anaphylaxis, gel diffusion methods, as detailed below, will be performed to see if antisera obtained against "old" tissues contain additional antibodies not present in the antisera obtained against "young" tissues. Finally, if the above tests on isolated sera prove to be negative, the procedure involving active systemic anaphylaxis in guinea pigs following desensitization with homologous and heterologous antigens will be tried as was done by Karaev.

1. Animals - Tissue Donors: As tissue and blood donors, genetically isologous rats will initially be utilized to avoid antigenic differences due to genetic factors. (It would appear, however, from the work of Karaev, who used tissues of random cows, that genetic factors did not affect his study. He notes that sera of different individuals of the same age, in a given species, had identical antigenic properties). The oldest animals in the colony will

be used as donors of old tissues. For young tissues, initially 3-4 week old animals and young adults (8-10 mo.) will be used.

Animals - For Antibody Production: Since rabbits are known to be good producers of antibody of the precipitating type, the animals from commercial sources will be initially used. (Specific Antigen-antibody immune precipitates, suitably purified can be a source of the antigen for characterization, etc.). If rabbit experiments prove to be negative guinea pigs will be immunized as was done by Karaev.

2. Antigens - Tissue Extracts: Karaev obtained positive results with saline extracts of tissues. These will be the sources of antigen for the present studies. A constant ratio of tissue (wet weight) to saline will be adhered to in order to avoid problems due to differences in antigen concentrations. In some experiments, the suitability of utilizing whole tissues for immunization will also be explored. Karaev employed the following materials or their extracts as antigens: Sera, spleen and liver.

The immunization procedure will consist of a series of four injections of increasing doses of antigen (0.1 to 1.0 ml q saline extract or 0.5 to 5. ml q alum precipitated proteins) into the marginal ear vein for four weeks. One week after the last injection the animals will be trial bled and if needed another course of immunization will be given until the presence of antibodies can be established.

Should saline extracts or alum precipitates prove ineffective as antigens, the extracts emulsified in complete Freund's Adjuvants will be employed. In this case, injection of the emulsion will be carried out once a week intradermally or in foot pads. (Adjuvant Mixture: Bayol F and arlacel C in a 9:1 ratio + killed TB)

3. Antisera: The blood will be collected under sterile conditions, sera separated in the cold, decomplemented and stored at low temperatures.

4. Serological Tests:

I. Gel Diffusion Analysis: The double diffusion technique of ouchterlony has proved of great value in characterizing antisera. In this technique antigen and antibody are allowed to diffuse towards each other in an agar medium. If precipitating antibodies are present a precipitin band is formed when antigen and antibody coalesce in optimal proportions.. If more than one antigen-antibody system is present, several precipitin bands are formed representing the minimum number of antigen-antibody systems present. In the present study utilizing crude tissue extracts as source of antigens, several precipitin bands can be expected. One can obtain an idea of the complexity of the system from the number of these bands. If one of the bands is shown to be specific for the antigen in question, the band will be isolated from the agar for further study.

II. Precipitin Tests: Specific immune precipitates can also be obtained by adding appropriate proportions of antigen and antibody. Quantitative precipitin techniques as developed by Heidelberger will be employed for estimating the amount of antibody.

III. Complement Fixation: Complement fixation test offers an elegant method for the detection as well as estimation of antibody. To appropriate dilutions of mixtures of antigen and antiserum, guinea pig serum (source of complement) will be added. After incubation, sensitized sheep red cells will be added and the hemolysis occuring under defined conditions will be determined. The difference in the hemolysis caused by control (antigen + normal rabbit serum) and experimental mixtures (antigen + antiserum) gives a measure of the antibody.

IV. Immuno-Electrophoresis: The method of Grabar and Williams combining electrophoresis in agar with double diffusion technique of ouchterlony has proved to be extremely useful to immunologists for detecting and identifying antigen-antibody systems. The antigen mixture is first separated on agar by electrophoresis. Antiserum is then allowed to diffuse in gel perpendicular to the axis of electrophoretic migration. Each constituent forms a precipitin arc with its corresponding antibody which can then be defined by its position along the axis of migration. The number of lines obtained represents the minimum number of constituents in the mixture. The electrophoretic plates can be washed free of non-specific proteins, stained and photographed.

V. Passive Systemic Anaphylaxis: This test will be carried out in guinea pigs. The animals will be given intra-peritoneal injection of the appropriate dilution of antiserum. After 48 hours the antigen will be injected intracardially. Reaction of the tissue bound passively transferred antibody with antigen results in typical anaphylactic symptoms, usually fatal. This affords one of the most sensitive methods of detecting antibody.

VI. Passive Cutaneous Anaphylaxis: Ovary's procedure enables the detection of antibody levels as low as 0.003 mg ab N/ m/. Suitably diluted antisera (0.1 ml) will be injected intradermally into the backs of guinea pigs; at different sites. After 3-4 hours the animals will be challenged with an intracardial injection of the antigen mixed with 1% Evans Blue Dye in normal saline. Changes in tissue permeability mediated by the antigen-antibody interaction will cause a blueing of the antibody site. The size of blueing can be correlated with antibody content.

VII. Active Systemic Anaphylaxis. If the rabbit proves to be unsuitable as antibody producer, guinea pigs will be immunized against the tissue extracts. After 3-4 weeks of immunization, the animals are challenged with an intracardial injection of the antigen to produce active systemic anaphylaxis. For determining qualitative differences in antisera obtained against old and young tissues reciprocal desensitization procedures will be carried out prior to anaphylaxis experiments. For this, the animals immunized

with old tissue antigens will be injected with increasing doses of antigens of young tissues and finally challenged with old tissue antigens. The desensitization procedure will neutralize all the antibodies common to antigens of young tissues, leaving in circulation only the antibodies directed against any typical antigens of old tissues not present in young tissues. Similarly animals immunized against young tissues will be desensitized with antigens of old tissues and challenged with the antigens of young tissues.

5. Protein Estimation and Characterization: Many of the immunochemical procedures utilize classical biochemical techniques for protein characterization and estimation. These will include fractional precipitations, centrifugation, ion-exchange chromatography, gel-filtration, lyophilization, and electrophoresis on paper, agar and starch. The estimations will involve Kjeldahl nitrogen determinations, Folin-Wu, Biuret and Ninhydrin colorimetric procedures and ultraviolet absorption spectrophotometry.

Major Findings: A literature survey has been made on; a) Effect of age on immunological phenomena, and b) Role, if any, of immunological factors in aging processes. The result of this effort is the preparation of a review article entitled "Growth, Aging and Immunological Phenomena". The research proposal is based on this review of the literature.

Course of Project: The research program outlined will be initiated as the necessary resources can be provided.

Serial No. HD-AG32
1. Gerontology Branch
2. Comparative Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on Mitochondrial Energy Metabolism

Previous Serial Number: NHI-170

Principal Investigators: D. R. Sanadi (90% time)
R. L. Pharo (100% time)
K. W. Lam (100% time)
L. A. Sordahl (100% time)
J. B. Warshaw (100% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	6.9
Professional	4.9
Other:	2.0

Project Description:

Objectives: The principal aim is to dissociate and purify the components of the mitochondrial oxidative and energy coupling reactions. Reconstitution of the system with the discrete components of known function is important for a clear understanding of the mechanism of the energy-transduction process.

Major Findings: The research of the current year has centered on the resolution and purification of some of the enzyme components of the cardiac mitochondrial oxidative phosphorylation system which functions at the site of NADH oxidation. Three of the separated components have been studied intensively.

Coupling Factors: Submitochondrial particles which have been depleted by treatment with urea(urea-particles) carry out electron transport from substrates to oxygen unimpaired but have severely reduced efficiency in P/O and other energy-linked reactions. We had described previously the partial purification of an enzyme (coupling factor tentatively called Factor A) which restored P/O and the related partial reactions when added to the urea-particle. Factor A has been purified further to near homogeneity by the criteria of electrophoresis on cellulose acetate strips and on polyacrylamide disc electrophoresis. The additional purification steps involved chromatography on hydroxylapatite and ammonium sulfate fractionation. Factor A has low ATPase activity (1 to 2 μmoles ATP hydrolyzed/min/mg protein)

which can be stimulated over 20-fold by incubation at 43° at pH 8.5. The ATPase activity is insensitive to oligomycin. On the basis of its ATPase activity on heat-treatment, it is believed that Factor A is the terminal enzyme in the energy-transfer sequence, and is concerned with phosphorylation of ADP.

In the course of the purification of Factor A, it was found that crude preparations allowed a greater stimulation of energy-linked reactions in the urea-particle than did the purified Factor A. Using as an assay system the additional stimulation of ATP-dependent NAD reduction by succinate (reversed oxidative phosphorylation), obtained over and above that given by the urea-particle saturated with Factor A, Dr. K. W. Lam has purified a second factor. This work was difficult because of the small margin of additional activation above an already high level of activity. The new factor, tentatively called Factor B, has been purified approximately 75-fold by sequential ammonium sulfate fractionation, chromatography on DEAE-cellulose, then on hydroxylapatite, ammonium sulfate precipitation and finally rechromatography on DEAE-cellulose. It is completely separated from Factor A. It stimulates reversed oxidative phosphorylation (as above) at microgram levels. In a different type of phosphorylation-deficient respiratory particle (ammonia-particle), Factor B restores net P/O coupled to the oxidation both succinate and NADH. It has no ATPase activity even after exposure to elevated temperatures.

The results allow the conclusion that multiple soluble coupling factors are involved in oxidative phosphorylation at each site and that the factors do not appear to be site-specific. Apparently, the same set of factors operate at each of the three phosphorylation sites.

NADH-Ubiquinone Reductase: Two flavoproteins, both with Q reductase activity, have been separated by chromatography on hydroxylapatite. Their further fractionation by ammonium sulfate precipitation and adsorption on DEAE-cellulose yield final preparations which appear essentially as single bands on cellulose acetate or polyacrylamide gel electrophoresis. Their mobilities are different so that distinction by electrophoresis has become possible. The two enzymes have different sedimentation velocities (S_{20} of 4.7 and 5.5) and different molecular weights by the sedimentation equilibrium method (69,000 and 87,000). The hydrodynamic properties were studied in collaboration with Dr. H. Edelhoch, NIAMD. The less stable, lower molecular weight Q reductase (QR1) has 0.61 moles FMN and 2.8 moles non-heme iron per 69,000 g. QR2 has 0.89 moles FMN and 4.1 moles non-heme iron per 87,000 g. The FMN content is probably less than the expected minimum molar ratio of 1 probably because the enzymes are somewhat unstable and may lose flavin during purification. The flavoproteins also contain bound acid-labile sulfur which can be liberated as H_2S on acidification.

Proposed Course of Project: The physical changes in Factor A after heat-activation of ATPase will be examined. The role of Factor B will be studied by means of partial reactions including effect on ATPase of Factor A, ATP-P_i or ATP-ADP exchange. The chemical analysis of the ubiquinone reductase

flavoproteins and the mechanism of the catalytic reaction will be undertaken. Search for additional factors associated with the energy transfer sequence will be continued.

Honors and Awards:

D. R. Sanadi was invited to present a paper at the Gordon Research Conference on Energy Coupling Mechanisms in August, 1965.

D. R. Sanadi was invited to contribute two chapters to a reference volume on "Methods in Enzymology", (Ed.) R. Estabrook and M. Pullman.

Publications:

Andreoli, T. E., Lam, K. W., and Sanadi, D. R.: Studies on oxidative phosphorylation. X. A coupling enzyme which activates reversed electron transfer. J. biol. Chem., 240: (6), 2644-2653, June 1965.

Sanadi, D. R., Pharo, R. L., and Sordahl, L. A.: Non-heme Iron in NADH-Coenzyme Q_{10} Reductase in "A Symposium on Non-heme Iron Proteins" (Ed.): A San Pietro The Academic Press 1965, p. 429.

Sanadi, D. R.: Energy-linked reactions in mitochondria. In: J. M. Luck, P. D. Boyer, G. Mackinney, A. Meister, and E. E. Snell (Editors), Annual Review of Biochemistry. Annual Reviews, Inc., Palo Alto, Calif., 1964, Vol. 34, pp. 21-48.

Warshaw, J. B., Lam, K. W., and Sanadi, D. R.: Studies on Oxidative Phosphorylation. XI. Energy-dependent Reduction of Diphosphopyridine Nucleotide by ENE-DIOL Compounds in the Presence of Dyes. Archives Biochem. Biophys. (IN PRESS).

Warshaw, J. B., Lam, K. W., and Sanadi, D. R.: Studies on Oxidative Phosphorylation XIII. Uncoupling of Energy-Linked Reactions by 2-Thenoyltrifluoroacetone. Archives Biochem. Biophys. (IN PRESS).

Sanadi, D. R., Pharo, R. L., and Sordahl, L. A.: NADH-CoQ Reductase-Assay and purification in "Methods in Enzymology" (Ed.): R. Estabrook & M. Pullman, Academic Press (IN PRESS).

Sanadi, D. R., and Jacobs, E. E.: Assay of Oxidative Phosphorylation at the Cytochrome oxidase region (Site III), in "Methods in Enzymology" (Ed.) R. Estabrook and M. Pullman, Academic Press. (IN PRESS).

Serial No. HD-AG33
1. Gerontology Branch
2. Comparative Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Changes in Cellular Organelles and Metabolism with Age

Previous Serial Number: NHI-171

Principal Investigators: D. R. Sanadi (10% time)

Other Investigators: C. H. Barrows

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.1
Professional:	.10
Other:	1.0

Project Description:

Objectives: To compare the metabolic activity of old and young rats with particular emphasis on the terminal mitochondrial oxidation system.

Major Findings: The current phase of the studies on the comparative rates of oxidation of various ^{14}C-labeled compounds in young and senescent rats has been tentatively concluded with the analysis of DL-glutamic acid-3, 4-^{14}C and DL-alanine-3-^{14}C. No significant difference was observed in oxidation rates in 3 month, 12-14 month, and 22-24 month-old rats. The $t_{1/2}$ values and peak times for the elimination of the isotope as respired $^{14}CO_2$ were unusually long compared to the other previously studied compounds, presumably due to the use of racemic mixtures of the aminoacids rather than the physiologically active isomer.

The over-all results and conclusions from the study may be summarized as follows: Acetate-2-^{14}C is oxidized more rapidly in senescent rats than in adult rats. No significant age-associated differences in oxidatives rates were observed with several other compounds including acetate-1-^{14}C, Octanote-1-^{14}C, propionate-2-^{14}C, D-glucose-1-^{14}C, succinate-2-^{14}C, DL-alanine-3-^{14}C, and DL-glutamate-3-4-^{14}C.

Proposed Course of Project: The results have suggested that there may be an alteration with advancing age in the metabolic control mechanisms involving mitochondrial respiration and the extra-mitochondrial biosynthetic reactions.

Studies on the regulatory mechanisms, possibly involving changes in concentrations of intermediates, allosteric alterations of enzyme activity, and hormonal effects will be considered in future research.

Publications:

Sanadi, D. R., Barrows, C. H., and Crowder, S. E.: Oxidation of ^{14}C-labeled compounds in Rats of Different Ages, J. Gerontol.,

Corneo, G., Moore, C., Sanadi, D. R., Grossman, L. I., and Marmur, J: Mitochondrial DNA in Yeast and Some Mammalian Species. Science 151, 687 (1966)

Serial No. HD-AG34
1. Gerontology Branch
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on the Effects of Temperature and other Environmental Factors on the Aging Process

Previous Serial Number: NHI 179

Principal Investigators: M. A. Brock (20% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:
Total: .2
Professional: .2
Other:

Project Description:

Objectives: While golden hamsters are in deep hibernation, the erythrocyte life span is greatly extended and the concentrations of most erythrocyte phosphorylated glycolytic intermediates remain similar to the concentrations found in control hamster erythrocytes. Short-term (1 hr.) in vitro incubation at 4° C. of blood from control, cold-exposed and hibernating hamsters indicated that the erythrocytes from control and cold-exposed animals with normal body temperatures contained phosphorylated intermediates in concentrations different from in vivo levels. In contrast, marked changes were not apparent in hibernators' blood. This study further documents these differences in response to cold in vitro. In addition, it will attempt to show whether there is an active metabolic adaptation in erythrocytes which takes place at the onset of hibernation and if this is related to the observed increase in erythrocyte longevity.

Methods Employed: Blood was withdrawn from control and hibernating golden hamsters and kept at 0° C. for 0, 1, 2 and 3 hours prior to extraction with TCA. The extraction procedures and quantitation of intermediates using ion exchange column chromatographic methods were the same as previously described.

Major Findings: During in vitro incubation at 0° C. for 3 hours, the free glucose concentration in the plasma of hibernators remained nearly constant while that of the controls increased to 1.3 times the value found after 1

hour. In hibernators' erythrocytes, the free glucose concentration fell in 3 hours to half the 1 hour value. The control red cell glucose concentration decreased slightly. The precipitous drop in ATP concentration to 1/5 of the in vivo level of control erythrocytes after 1 hour cold exposure was again demonstrated. This low ATP concentration persisted during the 3 hours at 0° C. in vitro. In contrast, hibernators' red cell ATP concentration did not change over the 3 hour incubation period. ADP and AMP levels were virtually unchanged in both bloods, as were DPG and weak acid phosphates. The fructose diphosphate concentration increased to about 10 times in vivo levels during the 3 hours in vitro in both bloods. Non-fructose hexose diphosphate was unchanged in controls but completely disappeared in hibernators' erythrocytes after 3 hours. The only marked change in inorganic phosphate in the erythrocytes or plasma of either blood was an increase to about 1.3 times the control levels in the control plasma after 3 hours. The results indicate that in addition to the greater stability of ATP levels in hibernators' erythrocytes during in vitro cold exposure, there is apparently a difference in hexose metabolism as compared to red cells from control hamsters.

Proposed Course of Project: Concentrations of intermediates in control and hibernators' blood preserved in ACD for longer periods will be quantitated. The unidentified ultraviolet-absorbing materials will be studied in more detail. The glycolytic pathways will be studied using C^{14}-glucose and C^{14}-pentose incubated with blood at various temperatures.

Serial No. HD-AG35
1. Gerontology Branch
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Changes in Molecular Population During Development, Maturation, Aging, and Cellular Death.

Previous Serial Number: NHI 178

Principal Investigators: D. Hendley (100% time)
P. Gold (100% time) (E.O.D. 9/23/65)
B. Strehler (50% time)
D. Rubinstein (50% time)

Other Investigators: N. Anderson
W. Forbes

Cooperating Units: Veterans Administration Hospital, Baltimore, Md.
Baltimore City Hospitals
University of Waterloo, Ontario, Canada

Man Years:
Total: 6.28
Professional: 2.78
Other: 3.5

Project Description:

Objectives: The description of the occurrence and properties of molecular species during various phases of the life cycle including development, aging, and cellular death. Work in this area includes studies on the properties of age pigments, lipid changes, during cell death, particularly as a result of myocardial infarction, changes in phosphorylative capacity during aging and as a consequence of ischemia factors influencing oxidative phosphorylation, differences among cells in their compliments of s-RNA species and respective activating enzymes and the effects of serum from organisms of different ages on the growth of cells in artificial media.

Methods Employed: General biochemical techniques, tissue culture, and electronmicroscopy.

Major Findings: (1) Major unknown components have been obtained from human age pigment which have polarities between fatty acids and triglycerides and which contain neither acidic nor ester groups. The lipids of lipofuscin

preparations were found to have a higher proportion of nonpolar lipids to phospholipids than do mitochondria.

(2) Lipid components of myocardial tissue during infarction have been extracted and chromatographed by thin layer chromotography. No major changes during infarction were found by the techniques employed suggesting that the major differences observed histochemically are a result of the redistribution of pre-existing lipid pools.

The loss of phosphorylative capacity during cardiac ischemia has been studied on mitochondria obtained from such tissues. The capacity to phosphorylate decreases rapidly within 7 min. of the onset of anaerobic conditions in mouse heart muscle. This decline in phosphorylative capacity parallels the loss of ATP occuring during the same treatment.

In order to study phosphorylative capacity of minute amounts of tissue we have adapted the firefly luminescence system to the continuous measurement of ATP production by phosphorylating mitochondrial preparations. It was found that mitochondria are very susceptible to dilution, and that a concentration greater than about 0.15 mg of mitochondrial protein/ml is necessary for phosphorylation to take place.

However, the addition of serum albumin to diluted mitochondria restores phosphorylation to a high level. It has been shown that the effect of the dilution is not due to endogenous inhibitors present in the mitochondria preparation. Some of the inhibitory effects may be attributed to the presence of inhibitory materials in the diluting medium. The identification of these materials is presently being undertaken.

(3) The firefly luminescence system has also been employed in the measurement of phosphorylative capacity of mitochondria obtained from the liver, kidney, and heart of 12-month and 24 month-old rats. Preliminary results indicate substantially higher rates of phosphorylation in preparations (particularly from kidney mitochondria) from young animals than old.

(4) In order to determine whether differentiation and/or aging may be the consequence of the loss of specific transfer RNA species (or their corresponding activating enzymes) required to "read" certain messenger RNA codons (nucleotide triplets) a study of differences in activating enzymes and s-RNA species obtainable from different specialized tissues has been undertaken. Since almost all amino acids are coded by from 2 to 6 different codons, the possibility exists that a different complement of s-RNA's or activating enzymes in different specialized tissues, may favor the selective synthesis of different groups of specialized proteins.

Because of the large and obvious differences in the kinds of proteins synthesized by various differentiated cell types of multi-cellular organisms, initial studies are directed at the identification of potential differences in the types of transfer RNA or activating enzymes in heart, liver, and reticulocytes of the rabbit.

Using the incorporation of C^{14} or Tritium labelled amino acids into acid insoluble products in the presence of transfer RNA, ATP, and activating enzymes, it was found that unfractionated enzyme preparations catalyzed different amounts of synthesis depending on the tissue of origin. Liver enzymes were consistently more effective than similar preparations from heart. Studies with heart activating enzyme preparations fractionated on DEAE cellulose or calcium phosphate gel, however, suggested that the heart-liver differences are due to contaminating enzymes.

In delabelling experiments it was found that the capacity of enzyme preparations to remove label were not strikingly dependent on the tissue of origin regardless of the enzyme preparation used to install the label initially.

Proposed Course of Project: (1) In collaboration with Dr. Norman Anderson of the Oak Ridge Nat. Lab., the purification of age pigment particles by zonal centrifugation will be undertaken. In collaboration with Dr. Forbes of the University of Waterloo, Ontario, the physical properties including ESR spectroscopy of age pigments will be continued.

(2) The effect of various factors on the capacity of mitochondria to support phosphorylation will be continued, as will the study of the nature of the effect of serum albumin on oxidative phosphorylation.

(3) The effect of age on phosphorylative capacity of mitochondria will be studied in detail employing newly derived refinements in preparative and measuring techniques.

(4) The studies of S-RNA will be extended to enzymes activating other amino acids and to enzymes and RNA derived from other tissues and from animals of different ages. Chromatographic separation of transfer RNA will be applied in an effort to determine whether tissue and age differences exist in s-RNA patterns and related enzymatic activities.

Publications:

Hendley, D. D., and Strehler, B. L.: Enzymic activities of lipofuscin age pigments: comparative histochemical and biochemical studies. Biochim. Biophys. Acta, 99: (3), 406-417, June 22, 1965.

Serial No. HD-AG36
1. Gerontology Branch
2. Cellular and Comparative Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on the Relationship Between Proliferation, Regeneration, Differentiation, and Death of Cells in vitro and in vivo.

Previous Serial Number: NHI-177

Principal Investigators:
B. Strehler (50% time)
A. Raychaudhuri (50% time)
R. Hay (100% time)
R. Menzies (100% time) (E.O.D. 9/30/65)
D. Rubinstein (50% time) (E.O.D. 9/20/65)

Other Investigators:
R. Wilder
G. Weinstein

Cooperating Units:
Veterans Administration Hospital, Baltimore, Md.
Sinai Hospital, Baltimore, Md.
Dermatology Department, Miami University

Man Years:

Total:	5.65
Professional:	3.15
Other	2.5

Project Description:

Objectives: The various phases of the research reported in this project are concerned with the relationship between cellular properties at various portions of the life span and the aging process.

Methods Employed: Various tissue and cell culture techniques have been adapted and employed as well as appropriate specific radioactive tracer and radio-autographic methods.

Principle Findings: Primary cell strains of fibroblasts from chick embryonic skin and muscle have been studied in respect to their proliferative capacities during multiple subcultures in the manner of Hayflick. The cells exhibited a logarithmic growth rate during the first two to three weeks in vitro, followed by a progressive decline to a static state. The total number of generations was limited to 15-20 as compared to 40-60 observable with human embryonic lung. Cell saturation density declines progressively during

subcultivation. Although the ratios of RNA/DNA and total lipid/DNA are decreased in cells in the growth decline phase, protein and carbohydrated content do not change significantly.

A reproducible method which permits the establishment of differentiated myotubes in vitro has been developed as well as a quantitative micromethod to measure myogenesis which uses the firefly luminescence assay in the measurement of creatine kinase activity. Optimal conditions for muscle formation using skeletal muscle have been determined. Embryo extract markedly enhances myogenesis and a biocarbonate buffer (pH 7.4-7.8) containing less than 10% serum is required. Such cultures can be obtained only from embryos less than 14 days of age.

Preliminary results indicate that sera from old donors inhibit growth of chick embryo cells to a greater extent than sera from young individuals.

Techniques have been developed which have made possible the cultivation of insect embryonic cells in vitro. When grown on an unsupplemented medium, these cells show no division but remain viable for 5 months. However, when pupal or larval extracts are added the cells produce fibroblast-like processes and increase in size and number.

Bone marrow cell suspensions from young and old donor rats were studied in vitro with respect to their capacity to synthesize DNA and RNA. In contrast to the findings of Wulff on nondividing cell lines, marrow cells from young animals show a consistent significantly higher rate of incorporation of p^{32} into RNA. A slight increase in DNA synthesis was also observed when cells were incubated in identical media. The effects of both stimulatory and inhibitory factors present in sera from young and old animals are being studied.

A test of the applicability of the Hayflick observation on limited cell proliferative capacity in vitro to replenishing cell lines in vivo has been undertaken in collaboration with Dr. Weinstein of the University of Miami School of Medicine. Radioautographs of human skin biopsies have been prepared at various time intervals after pulse labelling with tritiated thymidine. An evaluation of the fate of sister labelled cells should furnish a critical test of the Hayflick hypothesis.

Proposed Course of Project: The work under way will be continued.

Serial No. HD-AG37
1. Gerontology Branch
2. Cellular and Comparative physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on the Comparative Physiology of Aging

Previous Serial Number: NHI 183

Principal Investigators: M. A. Brock (80% time)
A. Raychaudhuri (50% time)

Other Investigators: None

Cooperating Unit: Veterans Administration Hospital
Baltimore, Maryland

Man Years
Total: 1.74
Professional: 1.3
Other: .44

Project Description:

Objectives: The hydranths of the colonial coelenterate, Campanularia, function as adults for 5-10 days and then undergo regression. This study is designed to define the morphological sequences during the aging and regression of these hydranths and to clarify the role played by lysosomes and lysosomal enzymes in regression. The project is also concerned with the description of histochemical changes in the female reproductive system of the flour beetle, Tribolium confusum, during the aging process and the correlation of these changes with the functional capacity of these organs. Finally, the project includes studies on the histochemistry of the pituitary of young and old rats.

Methods Employed: Histochemical procedures, microchemical assay for enzymes, and electronmicroscopy.

Major Findings: (1). Histochemical data has shown that the activity of acid phosphatase, a lysosomal enzyme, increases with advancing age of Campanularia hydranths. Microchemical findings during this year have shown that the free and total acid phosphatase activity is greater in 8-day-old than in 2-day-old hydranths when expressed as units of enzyme per hydranth. However, the protein concentration is greater in the older individuals and

acid phosphatase activity per unit total protein is not significantly different between young and old hydranths.

(2) Histochemical studies of age-related changes in the flour beetle have shown a heavy localization in the ovaries and a moderate concentration in the lateral oviducts, common oviduct, spermatheca, and spermathecal glands. A substantial decrease of these enzymes in all parts of the female reproductive system at 8-9 months has been observed. A slight esterase activity is present in the young ovaries but completely absent in the old. However, esterase activity increases in old oviducts, spermatheca and spermathecal glands.

(3) Histochemical studies on the pituitary of young and old rats have revealed a marked increase in neutral lipids during aging of the neurohypophysis whereas phospholipids acidic groupings, tyrosine content, and PAS positivity apparently decrease.

(4) A number of notable changes have been found during aging in the histochemical properties of the firefly lantern and a life table for this insect has been constructed from collections in the wild maintained in the laboratory. The longest-lived individual under laboratory conditions survived for 54 days and the mean lifespan was about 22 days.

Proposed Course of Project: The studies under way will be continued.

Publications:

Press, G., Raychaudhuri, A., and Strehler, B. L.: Histochemical Changes in the Firefly Lantern During the Aging Process. J. Geront. 21: (1), 13-21, Jan. 1966.

Serial No. HD-AG38
1. Gerontology Branch
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Fractionation of aging connective tissue

Previous Serial Number: none

Principal Investigator: H. R. Elden (1/4 time)

Other Investigators: none

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	1.75
Professional:	.25
Others:	1.5

Project Description:

Objectives: Collagen has been called "soluble" and "insoluble" depending upon its ability to dissolve in certain solvent, or combinations and successions of solvents. Few of these measurements show the serial depletion of collagen from whole tissue by repeatedly extracting samples with the same 'solubilizing' agent. In the study described herein, the objective is to measure the total quantity of collagen which is depleted from whole tissue by serial reaction with but a single solvent. In this manner, one will more rationally define as soluble that portion which dissolves transiently, and the insoluble fraction will be accepted as a residue exhibiting constant or very low solubility.

Methods Employed: Samples of aging rat dermis and rat tail tendon are heated to 60°C. in water. At selected time intervals the quantity of protein dissolved is quantitatively determined. Three series of measurements were conducted on samples of dermis whose ages were 4-6 weeks, 6 months, 9 months, and 12 months. Fresh solvent was added repeatedly at fixed intervals of extraction time, e.g., 15, 30 and 60 minutes in three separate studies. The number of repeated extractions ranged from 25 (15 minute intervals) to 7 (1 hour intervals). This number and time of serial extractions were sufficient to cover the transient extraction of collagen.

Major Findings: The concentration of collagen present in various extracts decreased rapidly with aging; however, there were changes in serial extractability which showed transitory maxima in young age groups. Concentration decreased non-linearly with serial extraction until it dropped to just perceptible levels. The remaining non-extractable collagen was dissolved by autoclaving. The accumulative percentage of collagen isolated by repeated extraction decreased with aging, while the percentage remaining increased with aging. Comparable results were found when solubilization was measured on the basis of nitrogen, hydroxyproline, or tyrosine assays.

Proposed Course of Project: The fractionated samples of aging dermis and tendon are being subjected to electrophoretic analysis on a crylamide gel. This will give a refinement in component distribution on the basis of molecular weight. Next, the number-average molecular weight of these fractions will be determined by automatic osmotic pressure measuring techniques. Finally, the relationship between extractability and molecular weight will be used to describe a molecular weight distribution function of the polymerized collagen matrix of aging tendon and dermis.

Honors and Awards: none

Publications: None

Serial No. HD-AG39
1. Gerontology Branch
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Elasticity of aging connective tissue

Previous Serial Number: None

Principal Investigator: Harry R. Elden (50% of time)

Other Investigators: None

Cooperating Unit: Baltimore City Hospitals

Man Years

Total:	2.00
Professional:	0.50
Other:	1.50

Project Description:

Objectives: Stiffness of connective tissue has frequently been said to change with aging, although the mechanism of extensibility is not known nor is it clear what part of the life-span various authors consider as aging. This study deals with both these points. The objective is to establish a method for delineating the contribution of elastin and collagen to the overall elastic extensibility of connective tissue in tendons, dermis, and blood vessels. The findings then will be used to compare the influence of aging on crystalline collagen and the amorphous form which results when collagen is melted.

Methods Employed: The dependence of longitudinal force (F) on extension ratio (L/L_o), or strain, was measured with the Instron tensile testing apparatus. Stress was computed as F/W_o for tendon and tail blood vessels, where cross sectional area was approximated by the ratio of weight/length (W_o); dermal thickness was also evaluated as W_o by weighing a square piece of 1cm x 1cm dimensions.

Major Findings: A. Tendons. Elasticity data on aging rat tail tendons showed that F/W_o increased with the second-degree of strain increment, viz.,

$$F/W_o = K_c ((L/L_o) - 1)^2.$$

This empirical law reflects the fact that elastic (YOUNG'S) modulus for longitudinal extension does not remain constant, but it increases linearly with strain increment, viz.,

$$d(F/W_o)/d(L/L_o) = K_c((L/L)-1).$$

Further measurements indicated that K_c decreased exponentially with increasing W_o according to the equation,

$$\text{Log } K_c = A - B \log W_o.$$

A decreased and B increased during growth-development (0 to 3 months), but they did not change thereafter (aging). This was verified by data obtained on two strains of rats. The basis for this form of elasticity data is interpreted as due to the wave-form of collagen fibers in relaxed tendons. When a load is applied the easily stretched waves are transformed to inextensible straight forms; increasing load transforms more and more into the latter state thereby requiring an exponential increase in force. Reduction in K_c with increasing W_o, then indicates that large tendons are structurally imperfect compared to thinner ones. This imperfection is decreased during growth and development, but it reaches a constant level which does not change during subsequent periods of life that can be called aging.

B. Dermis. Extensibility of aging rat dermis is complicated by the fact that initial region of stress vs. strain is linear; however, at a critical strain (L_c/L_o) data abruptly became non-linear. It was found that the difference between measured non-linear and extrapolated linear data obeyed the second-degree equation of tendon extensibility. Three parameters were then defined to express the effect of aging on elasticity of dermis, viz., initial low modulus (K_E), critical strain (L_c/L_o), and non-linear high modulus (K_C). These were interpreted to represent the stiffness of elastin, slack or disorder in collagen fibers, and stiffness of the slack-free collagen fibers, respectively.

Dermal thickness increased with aging, but there was only a slight reduction in the three parameters of elasticity. Other than a marked hysteresis in L_c/L_o in the youngest age group (2 months), aging was not a significant factor in dermal thickness.

C. Blood Vessels. Rat tail artery and vein exhibited an initial region of linear extensibility followed abruptly by non-linearity. Their data were treated similar to that of dermis, and values of K_E, L_C/L_O, and K_C were evaluated. This analysis was also applied to data on the pressure-circumference relationship of aging human arteries in the literature.

It was observed that tail vessels behave analogously with their anatomically approximate tendons. They do not change greatly with aging, although more samples must be measured. Human arteries, on the other hand, showed continuous increasing values of K_E and K_C, while there was a steady decline in L_C/L_O beyond the 20-30 age group up to the 80-90 age group. Values obtained at the 0-10 and 10-20 age groups were least influenced by age.

Proposed Course of Project: These measurements will be applied to tendon,

blood vessels, and dermis obtained from aging rats and mice of various strain, and human tissue as well. Also, the molecular rearrangement of collagen during extension will be investigated with a sonic pulse transmitter in order to verify the hypothesis of order-disorder phenomena as a basis for the non-linearity. Since the changes with age in elasticity of crystalline collagen fibers is not uniform in all tissues, reasons for selection will be sought. In particular measurements will be made on the extensibility of fetal calf tissue (tendon, dermis, and blood vessels) as a function of anatomical location and gestational age. This will be followed by studies on the post-fetal growth, development, and aging.

Serial No. HD-AG40
1. Gerontology Branch
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Hydration of aging connective tissue

Previous Serial Number: None

Principal Investigator: Harry R. Elden (25% of time)

Other Investigators: V. Hlavackhova (100% of time) (E.O.D. 1/17/66) (Left 3/31/66)

Cooperating Unit: Baltimore City Hospitals

Man Years
Total: .45
Professional: .45
Other: .00

Project Description:

Objectives: Connective tissue has the capacity, and perhaps one of its most important physiological functions, to absorb large quantities of water. The objective of this study is to determine whether or not alteration in connective tissue water with aging can influence the permeation and diffusion of solutes and water in inter-cellular space. The mechanism of water absorption and permeation are investigated herein as fundamental aspects of this problem by determining the adsorption isotherm, rate of dehydration and compressibility of dermis.

Methods Employed: Fetal calf skin and aging rat skin were extracted with hot ethanol, dried to constant weight under vacuum, then cut into pieces weighing about 1.0 grams. The quantity of water absorbed at equilibrium hydration was determined by suspending samples over sulfuric acid-water solutions. The partial vapor pressure of water in the gas phase was varied from 0 to 1.0 by adjusting the concentration of sulfuric acid. Samples were weighed every 75 - 100 hours until they reached constant weight when equilibrated at 20° C. This required about 3 weeks when the gas phase was not agitated. Final data were plotted as grams of water/100 grams of dry sample versus partial vapor pressure. The surface area of one monolayer and energetics of sorption were calculated according to the B.E.T. theory of multilayer adsorption.

Dehydration velocity was determined by weighing samples in a specially

constructed apparatus. Dry nitrogen was blown over the sample at a delivery rate of 100 mls./min. at 20° C. while samples were weighed on an analytical balance.

Compressibility was measured with the Instron tensile testing apparatus using a compression strain gauge. Data obtained on 1 cm. diameter pieces were treated similar to that of extensibility.

Major Findings: Water adsorption isotherms were the usual sigmoid shape of multilayer adsorption; plots of data satisfactorily obeyed the B.E.T. theory at partial vapor pressures less than 0.75 as expected. Aging did not change the area of monolayer coverage nor did the energetics of adsorption change. Likewise, there were no abrupt changes in these parameters during fetal development of calf skins. Generally, it appeared that the quantity of water sorbed depended upon the thickness of sample, or the total hydroxyproline (collagen) deposited.

Rate of dehydration of abdominal skin was approximated by first-order kinetics, and a specific rate constant was calculated from the time-course of weight lost during the first hour. Dehydration of dermis did not change with age, i.e., both the quantity and rate of water lost were constant for samples of 2 to 12 months of age. Freshly dissected skin also did not change with age, even though reports show otherwise. Possibly this disagreement is due to difference in experimental procedure.

Compressibility and tensile extensibility are similar in form; an initial linear increase in force accompanies both compression and extension while there is followed abruptly at a critical strain (L_c/L_o) by a nonlinear increase in force. These data were separated into contributions of elastin and collagen by the method used for dermis. Characteristic parameters of elastin and collagen declined rapidly during early hydration time, but they rapidly became constant. The change in physical parameters was proportional to the quantity of water present in the dermis, thereby permitting one to follow the movement of water therein during rapid influx or efflux conditions.

Proposed Course of Project: Adsorption isotherm data indicate that maximum water sorption is regulated by the thickness of dermis. There are still a number of points to be considered before this measurement is considered as conclusively negative, i.e., adsorption isotherms of other types of aging skin, kinetic changes in heats of adsorption, and the heats of wetting. The study of dehydration of aging dermis is just beginning. It is planned to thoroughly investigate the mechanism of water transport in aging dermis. Possibly, the rate limiting step is diffusion controlled, or it might be that evaporation rate at the surface is rate limiting. These points will be studied in an attempt to interpret literature reports which show progressive decline in rate of dehydration. Compressibility measurements will be extended so that it will be possible to determine the elasticity of crystalline and amorphous collagen fibers in small samples. It will then be possible to determine the influence of aging on these properties, in addition to hydration, on samples which can be obtained from humans only by biopsy.

NICHD Annual Report
July 1, 1965 through June 30, 1966
Mental Retardation Program

The Mental Retardation Program has primary responsibility for research in the biological, behavioral and social aspects of mental retardation within the context of child health and human development. This responsibility embraces all factors - biological, social and behavioral - that influence subnormal development.

As of January 1966, the National Institute of Child Health and Human Development was supporting 67 research grants, totaling approximately $3.2 million for research directly related to mental retardation. In mental retardation research training activities, the Institute was supporting training grants, fellowships, and research career development awards, amounting to approximately $653,000 at that time.

Extramural Activities

During the past year, research activities continue to focus on three principal areas relating to mental retardation - inborn errors of metabolism, cytogenetics, and behavioral research.

Research Grants

Metabolic Defects: One of the largest programs in the country for detecting and studying inborn errors of metabolism associated with mental retardation is being conducted at the Children's Hospital of Los Angeles. Paper chromatography and other laboratory techniques are used to study blood, urine and cerebrospinal fluid from about 100 carefully selected patients each month. Clinicians refer patients in whom a hereditary condition is suspected, or who have mental retardation of unknown origin. Abnormal levels for amino acids, keto acids, sugars, indoles, phenols and other metabolites have been determined. The proportion of patients who show abnormal urinary metabolites has been between 5% and 10%. This high proportion may be attributed to careful preselection. Extensive studies have been conducted on histidinemia, cystathioninuria, maple syrup urine disease, idiopathic hyperglycinemia, galactosemia, phenylketonuria, and tyrosinosis.

Detecting phenylketonuria (PKU) in newborn infants is just the first step in the management of this metabolic disease, which usually leads to mental retardation if left untreated. Treatment of this condition consists of using as the main constituent of the diet a synthetic formula from which the amino acid, phenylalanine, has been removed. Phenylalanine, however, is essential for the growth and development of the child. Failing to maintain proper levels of this amino acid can lead to malnutrition and even death of the infant.

A team of investigators at Children's Hospital Research Foundation, Cincinnati, has developed a plan of treatment for PKU designed to permit each child to achieve good physical as well as mental growth. The plan

calls for individualized treatment for such children, frequent monitoring of serum and urine samples to insure a proper level of phenylalanine, and enlisting the parents' co-operation in trying to make the child's life as normal as possible. All seven children who were started on the diet in the first three months of life, and who remained on the treatment, were found to have achieved normal intelligence, which did not differ appreciably from their tested unaffected brothers and sisters. Those starting later were helped, but not as much.

The group has adapted paper chromatography to micromethods so that extremely small samples of blood and urine can be used for testing. The child's mother is shown how to take a small sample of blood from the infant's heel. Urine samples are collected on filter paper. The blood samples and dried filter paper are then sent to the hospital laboratory for the tests. These tests determine the amount of phenylalanine in the blood and the urine, and the amount of orthohydroxyphenylacetic acid in the urine. Children were found not to thrive when the serum level of phenylalanine went below 3 mg.%; the abnormal breakdown products were found when the level rose above 7 mg.%.

Several confirmatory tests are used before a definite diagnosis of PKU is made and therapy instituted. A number of factors can produce false positives in the currently used screening methods. Among these is the presence of elevated levels of tyrosine. This plan for managing PKU was worked out with support from an NICHD grant. This group is currently working on practical methods for diagnosing and treating other metabolic diseases.

Recent reports indicate that infants born to mothers having phenylketonuria may be retarded without measurable abnormality in phenylalanine metabolism. It is suggested that an elevated concentration of phenylalanine in the maternal blood may cross the placenta and in some way damage the fetal brain. Investigators from the University of Wisconsin found that amino acids are actively transported across the mammalian placenta, with concentrations in fetal blood being higher than those in the maternal circulation. Elevated concentrations of phenylalanine were induced by dietary means in the blood of pregnant Rhesus monkeys, and the active transport mechanism was evident at both normal and elevated concentrations. A normal placental process may thus magnify a maternal biochemical abnormality and produce a more profound disturbance in the fetus. These data help to explain how a biochemical disturbance in the pregnant female may have a profound effect on the developing fetus.

Experience at the University of Michigan has shown that giving women, who have given birth to a cretin, dessicated thyroid during their pregnancies can prevent cretinism in subsequent children. This extra thyroid helps the infants in the intrauterine stages of development; if their own thyroids are abnormal, they can be given the medication after birth. Though cretinism can be prevented by this technique according to these investigators, it would not be practical to give every pregnant woman thyroid, and a method of prediction that waits for the birth of the cretinous child is completely unacceptable. Attempts are being made to find the reasons why some mothers give birth to cretins, and what physiological mechanisms are involved. Answers to these questions would permit the development of feasible methods of

predicting which pregnant women are in danger of giving birth to cretins. A number of complex studies in laboratory animals and clinical observations in humans have demonstrated that the fetus gets the thyroid hormone it needs both from its own thyroid and from its mother. Cretinism possibly could thus result from a mild thyroid defect in both mother and fetus, or a severe defect in the mother alone.

Among the factors ruled out in the search for the cause of cretinism in the newborn have been hereditary differences in plasma proteins and the automimmune phenomena which have been implicated in some thyroid conditions. Investigators are currently testing whether thyroid-stimulating hormone from the pituitary crosses the placenta from mother to fetus and, if so, what role this might have in the development of cretinism.

A grantee in Japan described a hitherto unreported syndrome characterized by mental and physical retardation associated with an elevated serum level of folic acid. The two children with this syndrome were found to excrete large amounts of formiminoglutamic acid in response to histidine loading and are considered to have a deficiency of formiminotransferase in the liver. This investigator is now undertaking a survey of mentally retarded children in the hope of detecting additional cases and identifying carriers of this syndrome.

Cytogenetics: Ever since the discovery that mongolism is associated with chromosomal abnormalities, investigators have been seeking biochemical markers which would indicate the presence of the altered chromosomes. One grantee is using enzyme techniques developed for study of the inherited disease, galactosemia, in search for a biochemical marker in mongoloids. Several grantee investigators have been working with other enzymes on the mapping of gene locations. This Institute supports grantees at the University of Colorado; University of Wisconsin; Children's Hospital at Los Angeles; and Children's Hospital, District of Columbia, working on the mapping of human chromosomes.

One investigator is trying to develop a new technique which would detect hitherto undiagnosed chromosome anomalies. Cells in meiotic division are expected to show more subtle chromosome abnormalities than are visible in mitotic division. Studying meiosis, however, is more difficult than working with mitotic cells. Working with testis tissue from laboratory animals and humans, one grantee and his associates are experimenting with various culture media and are trying various combinations of hormones which are known to stimulate gamete formation. When the techniques have been completely worked out, small samples of testes tissue will be taken by biopsy from carefully selected male retardates over age 13 years. If the technique of meiotic chromosomal analysis can be perfected and does show more subtle chromosome differences than previously seen, the original promise of this field might be fulfilled.

Behavioral Research: Further knowledge about learning is essential in our understanding of mental retardation. In a study undertaken at the George Peabody College for Teachers in Nashville, Tennessee, two main methods are being utilized to improve the intellectually handicapped child's language

skills: a phonetic alphabet system with 64 letters, each representing a single speech sound, called an initial teaching alphabet (ITA); and the Peabody language development kit, a program of 200 prepared language lessons including specific language plans for each day with extra stimulation being supplied by the use of tape recorders, puppets, and other interesting materials. The first thing showing up in this project was the obvious - some teachers are better than others. One superior teacher, after six weeks of teaching with the ITA method - with culturally deprived first-graders who had not been in kindergarten - had her class performing at a level acceptable to some of the finest primary schools in the country. However, this project is testing whether the new techniques can work in the hands of average teachers, or even in those of teachers themselves handicapped by poor preparation in below-average school systems. Though it is too soon for definite judgments to be made on this ongoing work, the results so far indicate that children receiving special attention are doing much better than their controls in other Nashville schools.

A significant number of children are slow learners because of poor performance secondary to various sensory and motor deficits. The National Institute of Child Health and Human Development is supporting a long-term study at the Albert Einstein College of Medicine relevant to this problem. The work is based on the principle that each sensory system in the body - sight, hearing, taste, and touch - interacts with and is modified by other sensory systems. The investigators have developed a series of tests of auditory-visual, visual-haptic, visual-kinesthetic, and a combination of these integrations. The auditory-visual integration is tested by letting the child listen to a series of taps, and then having him match up what he hears with a series of dots on paper. The testing program showed that reading disabilities seem to reflect an interference with auditory-visual interaction. Performance on the auditory-visual (dots and taps) test is correlated with the reading level and with IQ, and possibly offers a culture-free test of intelligence.

An investigator at the University of Connecticut is working with retarded children, trying to find out how the attention response can be conditioned and how the retardate's ability to make this response can be measured. The investigator is now concentrating on an oculomotor response. If a light in one place is regularly followed by a light in another, the human subject will, without instructions, move his eyes to anticipate the occurrence of the second light. The light can be varied with respect to brightness, size, hue, and flicker rate. As a possible practical application, the eye-movement tests might provide valuable information on the attention-response capability of retardates. This information in turn would help in devising ways of breaking learning situations down to tasks simplified enough for children with varying degrees of mental retardation.

The Institute is supporting studies on the effects of environmental deprivation on central nervous system development. Recently a group from the University of California at Los Angeles has described studies with rats in which environmental conditions were sufficiently profound to alter brain weight and various biochemical systems. Rats maintained for 30 days after weaning under conditions of environmental complexity or enrichment differ significantly from their isolated littermate controls. The rats in the

enriched environment had heavier brains and greater acetylcholinesterase activity than the isolated rats.

Training Activities

The number of training grants, fellowships, and research career development awards have shown a twofold increase during the past year. Still there is a critical need for more biomedical and behavioral research workers whose primary interests lie in areas related to mental retardation. This need will become very acute when the mental retardation research centers become operational. It is estimated that 70 additional investigators will be needed in each Center within two years after the completion of a Center. With the assistance of the Child Development and Mental Retardation Training Committee, programming of training grants has been intensified by contacting a large number of scientists in the field to encourage them to develop small research training programs.

Mental Retardation Research Center Construction Grants

Public Law 88-164, Title I, Part A, authorized the appropriation of $26,000,000 over a four-year period ending June 30, 1967, for the purpose of constructing research centers to assist in finding the causes and means of preventing mental retardation, or in finding means of ameliorating the effects of mental retardation.

The primary objective of the Mental Retardation Research Center Program is to provide facilities in particular environments where a cohesive program of research and research training can be accomplished. Administrative responsibility for this Program is concentrated at the NIH, specifically, the Division of Research Facilities and Resources, working in close collaboration with the National Institute of Child Health and Human Development. The National Institute of Child Health and Human Development is the focal point for program consultation as well as research and research training projects to be developed in the completed Centers.

During Fiscal Year 1966, three new Research Centers were approved and awarded, bringing to eight the total number of Centers awarded since the initiation of the Program in Fiscal Year 1964. The three construction grant awards in Fiscal Year 1966 were made to the University of California at Los Angeles, Los Angeles, California; Children's Hospital, Boston, Massachusetts; and the University of Kansas, Lawrence, Kansas.

The Center at the University of California at Los Angeles will have as its major focus a combined biological and behavioral approach to the problem of mental retardation. The Research Program there envisions co-operative studies among the Departments of Psychiatry, Neurology, Pediatrics, Biochemistry, and other Divisions within the Medical School, as well as the Departments of Sociology and Psychology. Collaborative studies of a basic and clinical nature related to mental retardation will be undertaken. The Center will have rich resources of scientific consultation and clinical patients through close working relationships with the Neuropsychiatric Institute, the Brain Research Center, the recently approved University-

Affiliated Facility for training and demonstration under Part B, Title I of PL 88-164, and Pacific State Hospital.

The Children's Hospital Medical Center, Boston, Massachusetts, is closely affiliated with the Harvard Medical School. The Mental Retardation Research Center at Children's Hospital proposes an interdisciplinary research program directed toward the understanding, prevention and amelioration of the handicapped and the mentally retarded. The research team of this Center will include representation from experimental neurological sciences, behavioral sciences, genetics, metabolism and clinical research. A University-Affiliated Facility for the mentally retarded has been awarded to Children's Hospital Medical Center and will complement the programs of the Research Center as well as providing a source of ambulatory patients for clinically oriented research.

The University of Kansas Center will be composed of three geographically separate units linked administratively into a comprehensive, integrated program of research. One unit, at Parsons State School, Parsons, Kansas, will provide an institutional research base where basic research and applied research on institutionalized retardates can be conducted. Another unit at the Kansas University Medical Center, Kansas City, Kansas, will include studies of reproductive physiology, biochemistry, neurophysiology and fetal and neonatal pathophysiology. Studies on learning, languages, and social behavior will also be conducted there. The third unit is on the University campus at Lawrence, Kansas. Research there will feature basic child development studies with an emphasis on socialization, communication, and learning. Staffs of each of these units have had long standing commitments to research on mental retardation. The long-range plans of the Center administration envisions a broad, multidisciplinary attack on the problem of mental retardation through the co-ordinated efforts of the staffs of the three units.

In addition to the three new Centers awarded in Fiscal Year 1966, two other Center applications were recommended for an award. These are at the University of North Carolina, Chapel Hill, North Carolina; and the University of Chicago's Wyler Children's Hospital, Chicago, Illinois. The latter has been recommended for approval of moveable equipment to aid in a program of research involving cytogenetics, neurologic investigations, experimental embryology, virology, and biochemistry. These Centers will be funded in Fiscal Year 1967.

Direct Research

Three Branches and one Laboratory have been organized to carry out direct research activities in mental retardation. These include a Clinical Research Branch, a Laboratory of Biomedical Sciences, a Behavioral Sciences Branch, and an Epidemiology Branch. The last two Branches are not operational as yet.

Two Sections will be included in the Clinical Research Branch - the Children's Diagnostic and Study Unit, and an Inpatient Diagnostic and Study Unit. Plans are now being formulated for the Inpatient Unit. The Children's

Diagnostic and Study Unit is operational and is located at the National Naval Medical Center.

The purpose of this Outpatient Clinic is to provide a program of clinical research and a source of case material for basic research in the biomedical and behavioral aspects of mental retardation. Currently two new patients are being evaluated each week by a multidisciplinary team. The Clinic team is interested in studies on learning. A small nursery school is associated with the Clinic and will provide an ideal setting for studies of learning, motivation, and teaching procedures.

Specific studies are being carried out on improving attending behavior in preschool retardates; behavioral assessment of neonates; informational discrepancies by parents; behavioral assessment in a free-play situation; and operant conditioning audiometric techniques.

A Laboratory of Biomedical Sciences is using borrowed space on and off the Reservation and has been collaborating with other Institutes on specific projects. Architects are now developing plans to renovate space for biomedical and behavioral research laboratories at the National Naval Medical Center. These laboratories will be located adjacent to the Children's Diagnostic and Study Unit to facilitate collaboration between clinicians and laboratory investigators. This facility will be ready for occupancy early in 1967.

Staff and Organization

An Assistant Program Director has been appointed to assist the Program Director in the administration of extramural activities. At a later date an Assistant Program Director for Direct Research will be added to guide intramural activities. By June 30, 1966, the Mental Retardation Program will have 41 employees including professional and supportive staff. Twelve will be attached to the Extramural Program.

Efforts to attract an epidemiologist to direct the Epidemiology Branch have been unsuccessful, although several new leads are being pursued.

Program Goals

The Mental Retardation Research Centers Program has made excellent progress in its third year of operation. Appropriated funds for Fiscal Years 1964, 1965, and 1966 have been exhausted. For Fiscal Year 1967, $6.0 million has been authorized; however, applications in excess of $8.0 million have already been approved or are pending review by the National Advisory Councils. Therefore, existing legislation will provide for 10 or 11 Research Centers.

There is a need for approximately 26 Mental Retardation Centers dispersed throughout the United States. Some will be large multidisciplinary Centers involving many colleges and departments of a university. Other Centers will be of a more limited scope, focusing on a particular problem of human development of high relevance to the mental retardation field in keeping with policy developed by the National Advisory Child Health and Human

Development Council.

To carry out this objective, legislation to extend and expand the Mental Retardation Research Centers Program will be needed next year. It is proposed that $12,500,000 will be required for Fiscal Years 1968, 1969, 1970, and 1971.

A major share of Federal support of research and research training to be carried out in these Centers will come from this Institute. Funds to implement research in each of these Centers will be required, and two Centers obtained this type of support during the past year. When the Centers become operational, it is anticipated that each Center will request approximately $800,000 for research support, and $150,000 for research training each year.

The Children's Diagnostic and Study Unit and the Biomedical and Behavioral Laboratories at the National Naval Medical Center will provide important facilities for the conduct of research. However, inpatient beds for long-term observations are necessary. The feasibility of acquiring an inpatient facility is now being explored.

Contract Title: Research Conference on Cognitive Processes and Models of Intellectual Development in Retardation

Contractor: The Woods Schools

Money allocated: $14,200.00

An understanding of the cognitive processes and of the elements of intelligent behavior in normal individuals may be crucial to an understanding of the pathology of thinking characteristics of many retarded individuals.

This Conference, held November 19-21, 1964, brought together behavioral scientists prominently identified with the field of cognitive theory with those actively engaged in behavioral and learning research with the mentally retarded. The aims of the Conference were to stimulate thinking and research activities with retarded populations based on the latest advances in cognitive theory in order that the processes of intellectual development and modes of thinking in this group might be better understood.

The Conference was co-sponsored by the Woods Schools, the American Association on Mental Deficiency, and the National Institute of Child Health and Human Development.

A report of the Conference was published as a monograph supplement to the American Journal of Mental Deficiency in January 1966, Volume 70, No. 4, under the title, "Cognitive Models and Development in Mental Retardation."

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Mental Retardation Program
Contract and Collaborative Research

Contract Title: Research Conference on the Prevention of Mental Retardation through the Control of Infectious Diseases

Contractor: The University of Texas, Southwestern Medical School, Dallas, Texas

Money allocated: $26,298

The President's Panel on Mental Retardation recommended in its report of October 1962 that there be a number of highly specialized international conferences to deal with special research problems on mental retardation.

In response to this recommendation, the National Institute of Child Health and Human Development, in co-operation with the NIH Staff Group on Mental Retardation, asked the Department of Pediatrics of the University of Texas, Southwestern Medical School, and the Children's Medical Center of Dallas to join them in sponsoring the first of these research conferences. The Conference will be held from June 9-11, 1966, at the Cherry Hill Inn, New Jersey.

The purposes of the Conference are to delineate the significant advances as well as problems of infectious diseases as they relate to the causes of mental retardation; to consolidate existing world knowledge about cause and control; to utilize experts in the fields to determine the extent infectious diseases cause, or are a potential cause, of suboptimal performance in the world's population; to determine what facts are unknown in these relationships in order to initiate research and application of services which will lead to the solutions of the existing and potential problems; and to consolidate these facts in the form of a highly readable monograph that would have broad impact and applicable value for researchers and practitioners.

This will be a small working Conference consisting of less than 40 distinguished scientists. Participants will be asked to prepare position papers on certain defined areas. These papers will not just review the past but will synthesize new knowledge to indicate the objectives of future research.

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Mental Retardation Program
Contract and Collaborative Research

Contract Title: Program of Regional Co-operation in Mental Retardation Research in Institutions in the Western United States

Contractor: Western Interstate Commission for Higher Education, Boulder, Colorado

Money allocated: $69,706

The Western Interstate Commission for Higher Education (WICHE) has demonstrated that, by sharing on a regional basis special facilities and programs in institutions for the retarded, it becomes possible for states to achieve much more difficult objectives than to achieve them alone. This contract is for that general purpose and has the following specific aims:

(1) WICHE, through its Joint Data Collection Project, has been involved in co-operative collection of epidemiologic data by 13 western institutions for the mentally retarded. This contract provides for expanding and capitalizing on this data collection project as well as improving its relevance to epidemiologic concerns.

(2) To stimulate the development of research activities at all western mental retardation institutions, utilizing to the utmost the special resources of each, within the limitations of each.

(3) To provide assistance leading to the development of manpower and technical skill available at each institution to carry out specialized research investigations.

(4) To develop a continuing network of communication between and among researchers which can lead to the development of co-operative research programs within states and across state boundaries, involving both universities and service institutions.

(5) To study the possibility of developing a system or systems of rapid communication of research activities and findings, to help decrease the "gap" between the development of new research findings and the testing and application of such findings in other settings.

The methodology is as follows: A Research Advisory Committee, a group of knowledgeable experts, will assist the WICHE project staff in examining data currently available as a result of the Joint Data Collection Project in Mental Retardation. These experts will provide advice in the biomedical, behavioral and social aspects of mental retardation, and will aid staff in expanding and improving the data collection questionnaire. WICHE staff will be responsible for the statistical development of this aspect of the research proposal.

During the first year of the project, an assessment will be made of each institution's specific interest in initiating a research program, personnel and facilities available to support the research and the specific content area to be developed. This information will be obtained by means of a questionnaire sent to all participating institutions. With this basic information in hand, research consultants who can contribute to the effective design and implementation will be made available to the institutions. The major emphasis in this effort will be the development of sound research, relevant to the needs and interests of each institution.

A directory of mental retardation research projects current in the western states will be developed and a system for continually updating this information will be established. The directory will be a ready source of knowledge about the specific areas of research being worked on in the West and the persons carrying out the work. It will be published periodically and given wide distribution among research and other interested persons not only in the West but nationally as well.

Small informal study conferences will be sponsored where small groups of researchers interested in or working in the same area are brought together to assist rapid transmission of knowledge and encourage the sharing of the regional resources for the most effective research planning and implementation.

Progress to date has been satisfactory. The Research Advisory Committee has been established and is functioning in accordance with plans. Staff to carry out the project has been recruited. Revisions and improvements have been made in the data collection forms. The number of state institutions participating in the data collection process has been expanded from 13 to 17.

The IBM cards and the "Population Census" forms used in the 1964 Joint Data Collection Project have been requested and received from Pacific State Hospital. This material on approximately 15,000 patients included in the 1964 data collection is being reviewed and evaluated for computer programming and analysis.

This project has considerable significance. The Report of the President's Panel on Mental Retardation notes: "The Federal Government should develop a comprehensive, continuing program for the collection and analysis of population statistics on the incidence, prevalence, and personal and socioeconomic characteristics of the mentally retarded." It is believed that the work of the Research Advisory Committee in expanding the scope and relevance of the Joint Data Collection Project in Mental Retardation will be a partial step toward fulfillment of this recommendation and thus have significance in implementing the recommendation of the Report.

The Report of the President's Panel states further, "The Panel recommends that high priority should be given to developing research centers on mental retardation at strategically located universities and at institutions for the retarded." In the West, this recommendation can best be implemented on a regional basis. There are many states in the western area of our nation without medical schools, without large universities, and with institutions

located far from large population centers. Some of these states could not support groups within their own boundaries, although they badly need the stimulation provided by such activities. For these states, the units referred to in the Panel's recommendation can therefore best be conceived of as regional units - a shared resource for several states.

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Mental Retardation Program
Contract and Collaborative Research

Contract Title: Inter-American Workshop on Mental Retardation

Contractor: University of Puerto Rico, Rio Piedras, Puerto Rico

Money allocated: $32,966

The NICHD shared with several agencies of the Department of Health, Education, and Welfare the costs for funding this contract, the purpose of which was to provide for the conduct of an Inter-American Workshop on Mental Retardation.

The Workshop, held in San Juan, Puerto Rico, October 17-23, 1965, had as its objectives: 1) the delineation of mutual areas of interest and need in the field of mental retardation among American countries; 2) to uncover potential areas of research activities and resources in Latin America that would be helpful to the United States; and 3) to determine the need for future international co-operation that will assist in the development of effective mental retardation programs in the Western Hemisphere.

Thirty-four official delegates from 14 Latin American countries attended the Workshop. Other official delegates from Puerto Rico, the United States and Canada also attended. Consultants attending the Workshop represented the Department of Health, Education, and Welfare, Pan American Health Organization, Agency for International Development, National Association for Retarded Children, Peace Corps, President's Committee on Employment of the Handicapped, American Association on Mental Deficiency, and the Inter-American Children's Institute.

The five-day program consisted of panel discussions addressed to the topics of prevention, management, care, and resources for training and research. Formal addresses and reports on status of mental retardation in Latin American countries were given.

The objectives of the Workshop were met to a large extent. The real value was in the exchange of information among the countries and the identification of resources in Latin America which might lend themselves to the research interests of United States investigators. Capable and knowledgeable persons in Latin America were identified, and steps were taken to assure a continuing program of information exchange.

A Follow-Up Committee on the Inter-American Workshop on Mental Retardation was established as a result of the Workshop. This Committee is responsible for issuance of the Workshop proceedings and co-ordination and implementation of Workshop recommendations which involve the Department.

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Laboratory of Biomedical Sciences
Mental Retardation Program

The mission of the Laboratory of Biomedical Sciences is to contribute to the Program's multidisciplinary investigation of mental retardation. Initially the Laboratory will emphasize biochemistry, cytogenetics, and neurophysiology. To this end, investigators from each of these disciplines have been recruited. The staff of the Laboratory now consists of 15 individuals (9 professional, 4 technical, and 2 clerical).

During the past fiscal year, some of the biochemical research has been located in Room 308, Building 4, NIAMD. This module has been occupied by Dr. Robinson since his transfer from NIAMD to NICHD in July, 1965, and will be vacated on July 1, 1966. The Cytogenetics Unit, headed by Dr. Matti Al-Aish, is located in the REEL Building at the National Naval Medical Center (NMMC). Dr. Frederick Maire joined the Laboratory in March and has begun his research work in neurophysiology in the Children's Diagnostic and Study Unit.

Plans for additional laboratory facilities are in the design phase. These laboratories will be located adjacent to the Children's Diagnostic and Study Unit at NNMC and will be ready for occupancy before the end of the next fiscal year.

To avoid a delay in staffing after completion of the laboratories, personnel have been recruited and are now engaged in collaborative investigation in other Institutes. This expedient also should help to increase our inter-Institute collaborative work in the future and thereby considerably augment our research program.

General Review of the Activities of the Laboratory

Projects in progress during the past year are enumerated below. They have been classified according to the major discipline involved, but many aspects of the work are interdisciplinary in nature.

Biochemical Investigations:

1. Pregnancy-Associated Plasma Proteins
2. Electrophoretic Variants of Leucocyte Alkaline Phosphatase
3. Inhibition Studies on Serum Cholinesterase
4. Development of a General Method for Detecting Peroxide-Producing Enzymes on Starch Gels
5. Hypercalcemia Study
6. Effects of Nutrition on Myelinization
7. Effects of Amino Acids on Myelinization
8. Evaluation of Methods of Therapy in Homocystinuria

Cytogenetic Investigations:

1. Cytogenetic Aspects of Mental Retardation
2. Studies on Human Chromosome Abnormalities
3. Alteration of Metaphase Chromosome Morphology and DNA Synthesis During Mitotic Cycle
4. The Role of Viruses in Chromosome Abnormalities in Spontaneous Abortions
5. Cytogenetic Studies of Viral-Transformed Hamster Kidney Cells
6. Clinical Cytogenetic Studies
7. Cytogenetic Studies of Human Abortuses
8. Chromosome Morphology in the Vitamin E Deficient Rat

Neurophysiology Investigation:

1. Serial Neurological and Neurophysiological Testing in the Diagnosis of Childhood Disorders

The thesis being advanced by the Laboratory, as is evident in the foregoing list of individual projects, is that an exhaustive effort should be made to describe as many of the mental retardation syndromes as possible in terms of modern molecular biology. This approach leads naturally to an examination of the sequence of events through which the genetic information of the chromosomes is utilized for biosynthesis of enzymes and structural macromolecules. The effect of an abnormal enzyme is frequently manifest as an accumulation of its substrate resulting in profound derangement of the normal pathways of intermediary metabolism. Two important aspects of these derangements must then be considered: 1) the effects of excess or deficiency of normal intermediary metabolites on cellular differentiation and organogenesis (particularly of the brain); and 2) the pharmacologic consequences of these excesses or deficiencies on nervous and mental function. Pursuant to the objective of describing mental retardation at the molecular, cellular, and organ levels of organization, the Laboratory is emphasizing genetics, developmental enzymology, intermediary metabolism, and neurophysiology.

In addition to its research activities, the Cytogenetics Unit has been responsible for the training of four individuals in the latest cytogenetic techniques during the past year.

Serial No. HD-MR1
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Pregnancy-Associated Plasma Proteins

Previous Serial Number: NIAMD-EBB-9

Principal Investigator: J. C. Robinson, M.D., Ph.D.

Other Investigators: G. Lee
J. E. Pierce

Co-operating Units: W. T. London, Institute for Cancer Research, Philadelphia, Pennsylvania
H. Marquez, University of Mexico, Mexico, D. F.

Man Years

Total:	10/12
Professional:	5/12
Others:	5/12

Project Description:

Objectives - Some of the objectives to be pursued in this project are: 1) to develop quantitative methods for the assay of the various pregnancy-associated plasma enzymes; 2) to determine whether the zymogram technique will be of value in differentiating recurrent choriocarcinoma from early pregnancy; 3) to ascertain the physiologic function of these enzymes; and 4) to evaluate the qualitative and quantitative distribution of these serum enzymes in abnormal pregnancies - i.e., pregnancies which eventuate in toxemia, miscarriage, premature birth, delivery of a malformed fetus, or delivery of a newborn who subsequently shows signs of mental retardation.

Methods Employed - The zymogram technique, in which sliced starch gels are incubated with substrate, was the principal method employed.

Major Findings - The major findings and conclusions are: 1) The "alpha-2 globulin of pregnancy" was found in the sera of nonpregnant females and in increased amounts in trophoblastic disease, during pregnancy, and during the administration of Enovid. The placenta, therefore, is not essential to the synthesis of this protein. Autoradiographs showed that the globulin does not bind I^{131} thyroxine. 2) The pregnancy-associated phosphatase and two cystine aminopeptidases, not being observed in patients with trophoblastic disease nor during the administration of Enovid, seem to be specific for pregnancy. Since they are absent from fetal blood, the placenta is the

probable site of origin of these enzymes. 3) Both Enovid administration and trophoblastic disease elicit the appearance of other aminopeptidase bands, one of which is similar in mobility to one of the pregnancy-associated cystine aminopeptidases. It can be differentiated from it by means of incubation with neuraminidase.

Significance to Biomedical Research and the Program of the Institute - Information has been gained concerning the biogenesis of proteins which occur in plasma during pregnancy, during hormone administration, and in trophoblastic disease. Observations have been made which possibly may aid in the differential diagnosis of pregnancy and recurrent choriocarcinoma.

Proposed Course of Project - Currently, serum samples from six patients with undelivered hydatiform moles are being examined for aberrant forms (or amounts) of the pregnancy-associated enzymes.

Honors and Awards: None

Publications:

1. Robinson, J. C., Pierce, J. E., and Blumberg, B. S.: The serum alkaline phosphatase of pregnancy. Am. J. Obst. and Gynec. 94: 559-565, Feb. 15, 1966.

2. Robinson, J. C., London, W. T., and Pierce, J. E.: Observations on the origin of pregnancy-associated plasma proteins. Am. J. Obst. and Gynec. (In press)

Serial No. HD-MR2
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Electrophoretic Variants of Leucocyte Alkaline Phosphatase

Previous Serial Number: NIAMD-EBB-8

Principal Investigator: J. C. Robinson, M.D., Ph.D.

Other Investigator: J. E. Pierce

Co-operating Units: P. Carbone and W. Rosse, Medicine Branch, National Cancer Institute, NIH
F. de la Cruz, Children's Diagnostic and Study Unit, Mental Retardation Program, National Institute of Child Health and Human Development, NIH

Man Years

Total:	11/12
Professional:	10/12
Others:	1/12

Project Description:

Objectives - The objective of this project is the characterization of isolated leucocyte alkaline phosphatase isozymes.

Methods Employed - The butanol procedure for solubilizing leucocyte alkaline phosphatase, devised by the principal investigator, was used. Starch gel zymograms of the extracted phosphatases were prepared.

Major Finding - After extraction of their leucocyte phosphatases, zymograms of 25 chronic myelogenous leukemia patients have been prepared. Analysis of the data is not complete, but striking deviations from the normal zymogram pattern are evident.

Observations on the starch gel patterns of leucocyte phosphatase variants have been extended to include other conditions in which quantitative alterations of leucocyte alkaline phosphatase have been reported. The following conclusions were made: 1) The displacement of the normal bands at the origin and at 1 cm. towards the anode has been detected only in chronic myelogenous leukemia. 2) The finding of phosphatase activity in the position between the normal fast (3 cm.) band and the two slow bands (origin and 1 cm.) is not specific for chronic myelogenous leukemia, being observed also in acute lymphatic leukemia, in one case of paroxysmal nocturnal

hemoglobinuria, in one patient receiving cortisone, and in pregnancy. 3) The fastest component (near 5 cm.) in chronic myelogenous leukemia is also not specific for that disease - it was detected in one of the patients receiving cortisone. 4) The fast band (near 4.5 cm.) in paroxysmal nocturnal hemoglobinuria has been observed in that disease only. 5) The zymograms of three cases of Down's syndrome appeared normal.

Significance to Biomedical Research and the Program of the Institute - Interpretation of these pronounced, qualitative modifications of leucocyte alkaline phosphatase cannot be made at present. It should be noted, however, with respect to the unique band (near 4.5 cm.) in paroxysmal nocturnal hemoglobinuria, that the suggestion has been made (by Lewis and Dacie) that there may be two populations of neutrophils in this disease - one normal, the other abnormal. It is possible, therefore, that the fast component is derived from an abnormal population of cells.

Proposed Course of Project - This work is to be extended to include substrate specificity studies on the phosphatase variants. In this way, information will be obtained which may give insight into the significance of the presence of the variant enzymes.

Honors and Awards: None

Publications:

1. Robinson, J. C., Pierce, J. E., and Goldstein, D. P.: Leukocyte Alkaline Phosphatase: electrophoretic variants associated with chronic myelogenous leukemia. Science 150: 58-60, October 1, 1965.

Serial No. HD-MR3

1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Inhibition Studies on Serum Cholinesterase

Previous Serial Number: None

Principal Investigator: J. C. Robinson, M.D., Ph.D.

Other Investigator: G. Lee

Co-operating Unit: T. Dublin, Epidemiology and Biometry Branch, National Institute of Arthritis and Metabolic Diseases, NIH

Man Years

Total:	5/12
Professional:	2/12
Others:	3/12

Project Description:

Objectives - The objective of this project was the evaluation of the agar diffusion test for detecting genetic variants of serum cholinesterase.

Methods Employed - The agar diffusion test was used for detecting cholinesterase variants. The results were checked by the spectrophotometric determination of fluoride and dibucaine numbers.

Major Findings - The results in the cholinesterase typing of 392 plasmas by the agar diffusion test were fully confirmed by the spectrophotometric determination of dibucaine numbers.

The main advantage of the agar diffusion test is the simplicity with which dibucaine variants can be detected. In our hands the test was of no value in detecting the UF phenotype.

The dibucaine number and the fluoride number were found to be inverse functions of temperature. The change in fluoride number per degree was roughly four times as great as the change in dibucaine number. Furthermore, the magnitude of the change of the fluoride number with temperature was greater with the U than with the UF plasmas.

By using the spectrophotometric method, the distinction between U and I dibucaine variants was apparent at 26°C, but the U and UF groups could not be

clearly separated. Since previous investigators have determined fluoride numbers at 21-25°C and since the distinction between U and UF is less marked when fluoride numbers are determined at elevated temperature, our failure to distinguish clearly these phenotypes may be related to the temperature effect. The necessity for precise control of temperature (preferably at near 20°C) in the determination of FN is emphasized by our data.

Significance to Biomedical Research and the Program of the Institute - The reliability of a simple, time-saving method for typing serum cholinesterase has been confirmed.

Proposed Course of Project - A manuscript reporting these results has been submitted for publication. Since the objective of this project - the evaluation of the agar diffusion test - has been accomplished, the project has been terminated.

Honors and Awards: None

Publications: None

Serial No. HD-MR4
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Development of a General Method for Detecting Peroxide-Producing Enzymes on Starch Gels

Previous Serial Number: None

Principal Investigator: J. C. Robinson, M.D., Ph.D.

Other Investigator: G. Lee

Co-operating Units: None

Man Years

Total:	5/12
Professional:	2/12
Others:	3/12

Project Description:

Objectives - The objective of this project was to devise a general method for detecting peroxide-producing enzymes on starch gels.

Methods Employed - A peroxidase-coupled enzyme system was employed to prepare zymograms of seven enzymes.

Major Findings - Experiments were conducted in an attempt to demonstrate on starch gels the pregnancy-associated diamine oxidase. Although the amount of this enzyme in serum is below the sensitivity of the peroxidase-coupled enzyme system employed, the system was found to be efficient in the detection of six other peroxide-producing enzymes against which it was tested. Since the method has potential in the study of isozymes, it seemed appropriate to develop it further in order to determine optimal pH values, substrate concentrations, and co-factor requirements. This has been completed, and the system has been used to detect the following peroxide-producing enzymes: L-amino acid oxidase, D-amino acid oxidase, galactose oxidase, glucose oxidase, xanthine oxidase, and kidney diamine oxidase. The dye employed in the system was found to react directly with ceruloplasmin. Thus, the system is a means for studying electrophoretic variant forms of at least seven enzymes, and theoretically it can be used to study any enzyme which forms peroxide as a reaction product.

Significance to Biomedical Research and the Program of the Institute - A method has been devised for studying electrophoretic variants of any enzyme which forms hydrogen peroxide as a reaction product. This method will permit the study of the differential specificity of some of these enzymes (e.g., the amino acid oxidases) to various substrates, and it has potential value in studying the tissue distribution of the enzyme variants during embryonic development.

Proposed Course of Project - After completion of a limited number of ancillary experiments with the system, the project will be terminated - the objective of the work has been accomplished.

Honors and Awards: None

Publications: None

Serial No. HD-MR5
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Hypercalcemia Study

Previous Serial Number: None

Principal Investigator: H. E. Wiltse, M.D.

Other Investigators: R. E. Cooke, M.D.
R. D. Rowe
S. Levin

Co-operating Unit: Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland

Man Years

Total	24/12
Professional:	12/12
Others:	12/12

Project Description:

Objectives - This study, intended to explore possible causes of the syndrome of mental retardation, supravalvular aortic stenosis, and distinctive facies, was initiated in 1964 and has continued to the present.

Methods Employed - A completed clinical survey of 15 young subjects with this syndrome has added to previously existing evidence linking the syndrome with infantile hypercalcemia. The survey results form the basis of a paper entitled, "Pathogenesis of the Supravalvular Aortic Stenosis Syndrome," which is in preparation.

A clinical study of a set of identical twins, who in infancy had hypercalcemia, has revealed the first known instances in which all components of the syndrome identified above have been present without the hallmark lesion of supravalvular aortic stenosis.

Major Findings - Two recently convalescent hypercalcemic subjects, age two, have been given a challenge dose of vitamin D under the conditions of a calcium balance test. Results of this experiment fail to support the existence of an intrinsic sensitivity to the hypercalcemic effect of vitamin D in these subjects; such sensitivity has been previously proposed as a possible cause for the disturbances in infantile hypercalcemia.

Substantial progress has been made with animal experiments in the following:

(1) Development of an experimental procedure for testing nephrotoxic agents for their effects in enhancing the hypercalcemic effect of vitamin D.

(2) Clarifying the role of acid-base disturbances in promoting inappropriate reabsorption of calcium ion by the renal tubule.

(3) Demonstrating a mechanism by which previous hypercalcemic injury can subsequently render an animal more susceptible to the hypercalcemic effect of vitamin D.

(4) Studying the hypercholesterolemic effect of toxic doses of vitamin D.

Significance to Biomedical Research and the Program of the Institute - A better understanding has been gained of a syndrome in which mental retardation is an element.

Proposed Course of Project - When vitamin D assays of the test and control sera have been completed, a manuscript for publication will be prepared, and the project will be terminated.

Honors and Awards: None

Publications: None

Serial No. HD-MR6
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Effects of Nutrition on Myelinization

Previous Serial Number: None

Principal Investigator: H. P. Chase, M.D.

Other Investigator: G. McKahn, M.D.

Co-operating Units: University of Colorado School of Medicine,
Denver, Colorado
Stanford University,
Palo Alto, California

Man Years

Total:	8/12
Professional:	8/12
Others:	0

Project Description:

Objectives - 1) to evaluate the effect of undernutrition from birth on myelinization; and 2) to try to determine if a "critical period" exists after which refeeding undernourished animals no longer results in correction of the decreased myelinization.

Methods Employed - Animals are divided at birth into large litters (poorly nourished) and small litters (well-nourished). The incorporation of $S^{35}O_4$ into myelin has then been followed for the first 90 days of life. Refeeding experiments, in which animals from the large litters were replaced into small litters, have been performed at two-day intervals from birth to age eighteen days.

Major Findings - The initial phase of the project is near completion, with results showing a decreased rate of myelin formation in undernourished animals. The refeeding experiments have shown inability for animals undernourished past 10 days of life to catch up to control animals in the rate of myelin synthesis.

Significance to Biomedical Research and the Program of the Institute - Early postnatal malnutrition has been shown to produce irreparable damage to the process of myelinization in the central nervous system.

Proposed Course of Project - Analysis of principal myelin lipids (cholesterol, cerebroside, cerebroside sulfate) is now underway - to further verify the results of $S^{35}O_4$ incorporation.

It would be of interest to evaluate other brain lipids in well-nourished and undernourished animals. Analyses of the fatty acid components of the various lipids for quantity, chain length, and saturation (by gas chromatography) are planned.

Honors and Awards: None

Publications: None

Serial No. HD-MR7
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Effects of Amino Acids on Myelinization

Previous Serial Number: None

Principal Investigator: H. P. Chase, M.D.

Other Investigator: D. O'Brien, M.D.

Co-operating Unit: University of Colorado School of Medicine, Denver, Colorado

Man Years

Total:	8/12
Professional:	8/12
Others:	0

Project Description:

Objectives - 1) to evaluate the process of myelinization as affected by various amino acids also found elevated in human diseases associated with mental retardation; 2) to determine if a period exists in rats injected with phenylalanine, after which the process of myelinization is permanently impaired; and 3) to study alterations in brain lipids as a result of phenylalanine injections.

Methods Employed - Various amino acids are injected into rats every eight hours from the time of birth. The incorporation of $S^{35}O_4$ into cerebroside sulfate of myelin is then measured in the test animals, as well as in control animals injected with saline. Lipid fractions of animals injected with phenylalanine are isolated with sephadex column chromatography and quantitated by standard methods. Experiments have also been carried out to demonstrate myelin synthesis after discontinuation of the phenylalanine injections in the rats of various ages.

Major Findings - The metabolic rate of synthesis of myelin has been shown to be greatly impaired in animals injected with phenylalanine. The study indicates that animals receiving phenylalanine after the first 10 days of life do not convert to the full rate of myelin synthesis seen in control animals.

Significance to Biomedical Research and the Program of the Institute - High plasma concentration of phenylalanine was found to inhibit myelinization in

the rat. The implication is that a similar inhibition may occur in phenylketonuria in human beings.

Proposed Course of Project - Other amino acids, including methionine, homocystine, histidine, and tyrosine are currently being injected into animals to determine their effect on the process of myelinization. Lipid analyses are being done on the brains of animals injected with phenylalanine.

Honors and Awards: None

Publications: None

Serial No. HD-MR8
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Evaluation of Methods of Therapy in Homocystinuria

Previous Serial Number: None

Principal Investigator: H. P. Chase, M.D.

Other Investigators: S. Goodman, M.D.
D. O'Brien, M.D.

Co-operating Unit: University of Colorado School of Medicine,
Denver, Colorado

Man Years

Total:	12/12
Professional:	12/12
Others:	0

Project Description:

Objectives - The objective of this project is to evaluate methods of therapy in homocystinuria.

Methods Employed - Four patients with homocystinuria have been studied to evaluate methods of therapy. Five means of therapy were attempted: pyridoxine, serine, cysteine, penicillamine, and dietary therapy. Methionine tolerance tests have been done on 15 family members, including four known heterozygotes. Blood samples were collected at regular intervals for fatty acid determinations as blood methionine decreased.

Major Findings - All samples have been collected, and studies are completed with use of the Beckman Amino Acid Analyzer on three of the four patients. Low methionine diet was found to be the only way to lower blood methionine levels. Detection of the heterozygote seems possible by means of the two-hour blood methionine level after a methionine load.

Significance to Biomedical Research and the Program of the Institute - Dietary therapy has been found effective in decreasing the blood methionine level in homocystinuria. The methionine loading test is a possible means for the detection of the heterozygote.

Proposed Course of Project - It is proposed that we 1) analyze the collected serum samples for fatty acids by gas chromatography, and 2) follow the

development of the patients while they are receiving dietary therapy.

Honors and Awards: None

Publications: None

Serial No. HD-MR9
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Cytogenetic Aspects of Mental Retardation

Previous Serial Number: None

Principal Investigator: M. Al-Aish, Ph.D.

Other Investigator: L. A. Goldsmith, M.D.

Co-operating Unit: Children's Diagnostic and Study Unit, Mental Retardation Program, National Institute of Child Health and Human Development, NIH

Man Years

Total:	15/12
Professional:	7/12
Others:	8/12

Project Description:

Objectives - A study of mentally retarded children and in some cases their parents, siblings, and other relatives, is in progress to determine the cytogenetic abnormalities responsible for mental retardation. This study includes investigations of chromatin bodies, drumsticks, dermatoglyphics, autosomes, sex chromosomes, etc.

Methods Employed - Short-term tissue cultures of peripheral blood and bone marrow are our present source of patients' chromosomes. However, we are planning to expand our facilities to include skin and other solid tissue cultures. This new approach, no doubt, will increase our knowledge of the genetic basis for mental retardation.

Major Findings - Our investigations include over 100 patients from the National Naval Medical Center, and over 20 children from the Children's Diagnostic and Study Unit, National Institute of Child Health and Human Development, Mental Retardation Program. Anomalies such as Down's syndrome, Turner's syndrome, Klinefelter's syndrome and Ph^1 chromosome were encountered. In addition to the classical syndromes mentioned above, the following abnormalities were observed: deletion of both arms of a single chromosome No. 18, trisomy of chromosome No. 17, fragments, rings, mosaicism, etc.

Significance to Biomedical Research and the Program of the Institute - This project is a part of the Mental Retardation Program's interdisciplinary study of mental retardation.

Proposed Course of Project - The project is to be continued.

Honors and Awards: None

Publications: None

Serial No. HD-MR10
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on Human Chromosome Abnormalities

Previous Serial Number: None

Principal Investigator: M. Al-Aish, Ph.D.

Other Investigators: None

Co-operating Unit: Children's Diagnostic and Study Unit, Mental Retardation Program, National Institute of Child Health and Human Development, NIH

Man Years

Total:	6/12
Professional:	4/12
Others:	2/12

Project Description:

Objectives - This project is specially designed for exhaustive studies on the chromosome complements of selected mentally retarded patients. Data from these cases are being accumulated for publication.

Methods Employed - Short-term tissue cultures of peripheral blood and bone marrow are our present source of patients' chromosomes.

Major Findings - The following cases have been identified and are under further study:

(1) A Turner's syndrome with 45/XO, 46/XX, 46/XX plus a fragment, and 46/X plus a ring mosaicism.

(2) A mentally retarded child with a deletion of both arms of a chromosome No. 18.

(3) A sex chromatin positive female with Turner's syndrome and 45/XO, 46/XX plus a fragment chromosome complement.

(4) A mentally retarded child with normal chromosome number: 46/XY with low percentage of cells with a fragment.

(5) A girl with Down's syndrome 47/XX (Trisomy 21) with fragments.

(6) A mentally retarded child with Trisomy 17.

(7) A mentally retarded child with 47/XX Trisomy 21 and fragments, deletions, and other anomalies.

Significance to Biomedical Research and the Program of the Institute - Knowledge of the developmental consequences of chromosomal abnormalities is increased by the work reported here.

Proposed Course of Project - It is proposed that the project be continued.

Honors and Awards: None

Publications: None

Serial No. HD-MR11
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Alteration of Metaphase Chromosome Morphology and DNA Synthesis During Mitotic Cycle

Previous Serial Number: None

Principal Investigator: M. Al-Aish, Ph.D.

Other Investigators: None

Co-operating Unit: Children's Diagnostic and Study Unit, Mental Retardation Program, National Institute of Child Health and Human Development, NIH

Man Years

Total:	4/12
Professional:	2/12
Others:	2/12

Project Description:

Objectives - The main objective of this project is to develop methods by which individual metaphase chromosomes can be distinguished.

Methods Employed - At the present time, this study is conducted on human chromosomes in vitro. Peripheral blood of patients and normal people has been cultured. Enzymes in varied concentrations were added to the blood culture before cell fixation. The time of enzymatic action in the cultures varied from a few minutes to a few hours.

Major Findings - Changes have been induced in metaphase chromosomes by the enzyme treatment, but analysis and interpretation of the data have not been completed.

Significance to Biomedical Research and the Program of the Institute - If this project is successful, methods will be available for identifying more of the individual chromosomes. This is possible at present for only a limited number of them.

Proposed Course of Project - It is proposed that the project be continued.

Honors and Awards: None

Publications: None

Serial No. HD-MR12
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Role of Viruses in Chromosome Abnormalities in Spontaneous Abortions

Previous Serial Number: None

Principal Investigator: J. D. Singer, M.D.

Other Investigators: J. L. Sever, M.D.
L. Carter

Co-operating Units: J. Sever, Section on Infectious Diseases, Perinatal Research Branch, National Institute of Neurological Diseases and Blindness, NIH
K. Sell, Tissue Bank, National Naval Medical Center, Bethesda, Maryland
J. Shaver, Department of Pathology, Suburban Hospital, Bethesda, Maryland
P. J. Schmidt, Clinical Center Blood Bank, NIH

Man Years

Total:	10/12
Professional:	5/10
Others:	5/12

Project Description:

Objectives - In view of the possible role which rubella virus and perhaps other viruses may have in relation to chromosome abnormalities, a combined clinical, viral, and cytogenetic study of early spontaneous human abortions is being undertaken.

Methods Employed - Culture of peripheral leucocytes and tissue culture are being employed to establish the karyotypes of the abortuses and of the family members. Viral cultures of the tissues of the abortuses and viral serological tests are also being employed.

Major Findings - The project was initiated only recently. Consequently, it is too early to attempt an analysis of the data.

Significance to Biomedical Research and the Program of the Institute - This study may reveal a relationship of viral infections in the mother to spontaneous abortions, and to fetal malformations.

Proposed Course of Project - It is proposed that the project be continued.

Honors and Awards: None

Publications: None

Serial No. HD-MR13
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Cytogentic Studies of Viral-Transformed Hamster Kidney Cells

Previous Serial Number: None

Principal Investigator: B. J. White, M.D.

Other Investigators: H. L. Cooper, M.D.
P. H. Block, M.D.

Co-operating Units: Human Genetics Branch, National Institute of Dental Research, NIH
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, NIH

Man Years

Total:	4/12
Professional:	4/12
Others:	0

Project Description:

Objectives - Simian Vacuolating virus (SV 40) is known to produce changes in appearance and growth characteristics (transformation) as well as chromosomal aberrations of hamster kidney cells in tissue culture. The aberrations include increased chromosome number as well as failure to establish a stable karyotype even after many successive passages of the initial infected culture. Because both adenovirus 12 and SV 40-transformed cells produce tumors (with abnormal chromosomes) when inoculated subcutaneously into young hamsters, a comparison of their effects on chromosomes of cells in culture is merited.

Methods Employed - Chromosome preparations of weanling hamster kidney cultures infected with SV 40, adenovirus 12, and a hybrid of SV 40 and adenovirus 12 have been made after early passages of the initial infected cultures. Modal chromosome number(s) will be determined and karyotype of cells with the modal number(s) will be prepared. This will be repeated again after numerous serial passages to further describe any karyotypically stable cell line which may have evolved and to detect any new cell lines.

Major Findings - The experiments are still in progress; no definitive findings have as yet been made.

Significance to Biomedical Research and the Program of the Institute - This project may provide new information on the mechanism by which viral infections produce developmental anomalies.

Proposed Course of Project - This study will be continued during the coming year.

Honors and Awards: None

Publications: None

Serial No. HD-MR14
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Clinical Cytogenetic Studies

Previous Serial Number: None

Principal Investigator: B. J. White, M.D.

Other Investigators: None

Co-operating Unit: Human Genetics Branch, National Institute of Dental Research, NIH

Man Years

Total:	4/12
Professional:	4/12
Others:	0

Project Description:

Objectives - Karyotypes of children with a number of clinical syndromes have been done to determine if any of these conditions are associated with a specific chromosomal abnormality.

Methods Employed - Short-term cultures of peripheral leucocytes and tissue cultures have been employed to determine karyotypes.

Major Findings - To date, normal karyotypes have been found in the following cases:

(1) Mandibulofacial dysostosis

(2) Chediak-Higashi disease

(3) Cleidocranial dysostosis

(4) Oro-facial-digital syndrome

(5) Chronic brain syndrome with aminoaciduria (amino acid analysis not completed)

The first case is described as functionally retarded due to defects of speech and hearing associated with the syndrome. The last three cases are also described as retarded in both speech and motor development.

Chromosome studies of the mothers of the patients with mandibulofacial dysostosis and Chediak-Higashi disease revealed no abnormality.

Karyotypes on another patient with cleidocranial dysostosis and a patient with odontogenesis imperfecta are in progress.

Significance to Biomedical Research and the Program of the Institute - The information gained in this project has increased our knowledge of a group of developmental disorders by showing that those syndromes studied were associated with normal karyotypes.

Proposed Course of Project - Karyotypes of unusual cases seen by clinicians of the Human Genetics Branch, National Institute of Dental Research, will be continued during the coming year.

Honors and Awards: None

Publications: None

Serial No. HD-MR15
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Cytogenetic Studies of Human Abortuses

Previous Serial Number: None

Principal Investigator: B. J. White, M.D.

Other Investigators: H. L. Cooper, M.D.

Co-operating Units: Human Genetics Branch, National Institute of Dental Research, NIH
Department of Obstetrics and Gynecology, D. C. General Hospital, Washington, D. C.

Man Years

Total:	4/12
Professional:	4/12
Others:	0

Project Description:

Objectives - Recent cytogenetic studies suggest that many human abortuses have abnormal karyotypes and that some of these abnormalities involve the X chromosome. Most of the chromosomal abnormalities found in cultures of human abortuses are also found in newborn infants or in adults in association with specific clinical syndromes and organ defects. These include Trisomy 21, Trisomy 13-15, Trisomy 17-18, and Turner's syndrome. The embryogenesis of the defects in these syndromes could be studied by karyotyping abortuses and examining histological preparations of the abortuses to determine their state of development.

Methods Employed - Tissues from intact human abortuses are being cultured to determine karyotype, and serial sections are made of the remainder of the abortus so that development of the various organ systems can be studied after the karyotype is completed.

Major Findings - To date, serial sections and chromosome preparations have been completed on two intact, spontaneous first trimester abortions. Review of the slides and preparation of the karyotypes will be done in the near future.

Significance to Biomedical Research and the Program of the Institute - This project will provide information on the association of chomosomal

abnormalities with spontaneous abortions.

Proposed Course of Project - The study of intact abortuses will be continued during the coming year. The size of the series will depend upon the number of abortuses available.

Honors and Awards: None

Publications: None

Serial No. HD-MR16
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Chromosome Morphology in the Vitamin E Deficient Rat

Previous Serial Number: None

Principal Investigator: L. A. Goldsmith, M.D.

Other Investigators: None

Co-operating Unit: National Naval Medical Center, Bethesda, Maryland

Man Years

Total:	10/12
Professional:	8/12
Others:	2/12

Project Description:

Objectives - The objective of this project is to test the hypothesis that, by lysosomal activation, vitamin E deficiency may produce chromosomal alterations in an intact animal.

Methods Employed - To test the hypothesis, a group of inbred Fisher rats were fed vitamin E deficient diets and control diets.

Major Findings - Vitamin E deficiency has been produced as evidenced by positive dialuric acid hemolysis tests, teeth depigmentation and histological signs of testicular degeneration. Mitotic chromosomes prepared from the bone marrow of the animals, and meiotic and mitotic chromosomes from the testes have been studied and to date do not show significant differences between control and deficient animals. These studies are still in progress.

Kidney lesions beginning with nephrocalcinosis and progressing to chronic renal disease and uremia have occurred in the vitamin E deficient rats and in those on a control diet. However, these lesions have not occurred in animals fed standard laboratory chow.

Significance to Biomedical Research and the Program of the Institute - These studies may yield information on the mechanism by which vitamin E deficiency produces a derangement of the reproductive process.

Proposed Course of the Project - The lesions and the diets are being analyzed for the etiological factors involved while further experiments are being performed to determine whether or not the lesions are reproducible.

Honors and Awards: None

Publications: None

Serial No. HD-MR17
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Serial Neurological and Neurophysiological Testing in the Diagnosis of Childhood Disorders

Previous Serial Number: None

Principal Investigator: F. W. Maire, M.D.

Other Investigator: None

Co-operating Unit: F. de la Cruz, Children's Diagnostic and Study Unit Mental Retardation Program, National Institute of Child Health and Human Development, NIH

Man Years

Total:	1/12
Professional:	1/12
Others:	0

Project Description:

Objectives - The differential diagnosis between the autistic or schizophrenic and the brain-damaged child remains a difficult problem. This diagnostic difficulty becomes compounded when it is realized that frequently the brain-damaged child manifests behavior of a psychological nature and, conversely, several of the workers dealing with childhood schizophrenia look upon this disorder as one of organic origin as opposed to schizophrenia occurring later in life. Moreover, the estimation of a child's true intelligence and degree of valid mental retardation when he is found to be in one of these diagnostically difficult groups becomes a rather thorny endeavor.

Working in the past with a large group of children composed of several diagnostic categories including mental retardation, brain damage and psychosis, some impressions were derived from carrying out extensive neurological and behavioral examinations, hinting that certain clusters of responses were useful in deriving a more definitive diagnosis. These findings especially appeared to take on meaning when they were derived from carrying out serial testing. Several of the observations have hithertofore not been reported, but there is a need to re-evaluate them with an enheightened element of sophistication and a research design lending itself to an improved mode of statistical analysis.

Methods Employed - Working with the Children's Diagnostic and Study Unit, these previous methods of neurological testing, some of original design, are being applied in serial fashion to the young patients being screened by that clinic. The tests, none of which cause the child any discomfort, include the following: multiple stimulation sensory testing in several combinations, double (analogous areas of the two sides of the body) stimulation, ease of eliciting perseveration through a skin sensory test, midline identification of the body tests, ascertaining aberrant patterns of unilateral skin temperature changes of the hands, vibratory sense changes (this has since been reported by another group and supports our original contention that schizophrenic patients show vibratory sense impairment which improves as does the patient), pupillary anisocoria seen in dim light only, graphesthesia testing, a map following test, and several others.

Efforts are being made to quantify the tests where possible. It is also hoped, that even though the patient in some respects acts as his own control in view of serial testing, at a later date to include matched normal controls.

Major Findings - Sufficient data to permit analysis have not been accumulated at this early date.

Significance to Biomedical Research and the Program of the Institute - The results of this study may aid in the differential diagnosis of brain damage and childhood schizophrenia.

Proposed Course of Project - As a parallel study of potential significance, upon completion of the Neurophysiology and EEG Laboratory, patients will have serial measurements made of their evoked cortical response to sensory stimuli as obtained through scalp electrodes. Adult studies utilizing this principle have indicated that psychotic patients show differences in their evoked cortical response patterns as compared to normals. These differences include an increased latency and a prolonged refractory period in responding cortically to a second sensory stimulus presented peripherally. These changes return to normal as the patient recovers. Such studies do not appear to have been applied to children having schizophrenia, mental retardation or brain damage and would appear to represent a fruitful approach for enhancing our diagnostic reliability in such cases, especially when using a serial approach.

Honors and Awards: None

Publications: None

NICHD Annual Report
July 1, 1965 through June 30, 1966
Children's Diagnostic and Study Unit
Mental Retardation Program

The Children's Diagnostic and Study Unit (CD&SU) is an outpatient facility where clinical research in biology, medicine, behavior, learning, speech and hearing, epidemiology, genetics as well as other types of investigations will be carried out on mentally retarded children. In addition, the Clinic will be used to train clinical and research associates as well as staff fellows. It will also be a source of clinical material for basic research. Collaborative studies will be carried out with the biochemistry, neurophysiology and behavioral sciences laboratories of the Mental Retardation Program.

A multidisciplinary team approach will be maintained in the clinical evaluation of patients. The Clinic population will be composed of children, six years old or younger, who fall under one of the following categories:

1. Those who appear or have been said to be developing slowly;
2. Those who appear or have been diagnosed as mentally retarded;
3. Those who appear or have been diagnosed as having learning handicaps or significant communication disorders; and
4. Those who appear or have been said to have birth trauma, neurological impairments or other injuries or symptoms which may be related to learning difficulties.

Normal controls, if indicated, will also be used in our studies.

On November 29, 1965, the Clinic staff moved to its present location in Building 125 at the National Naval Medical Center. Although the Clinic was officially opened on January 19, 1966, the first patient was evaluated on December 20, 1965.

In order to determine the proportion of mentally retarded children who are seen in the Pediatric Outpatient Clinic at the U. S. Naval Hospital, the Children's Diagnostic and Study Unit staff developed a case finding questionnaire which was mailed to families whose children were seen in the Outpatient Clinic between January 1, 1965, and July 31, 1965. Children who have been labeled as mentally retarded or developing slowly constitute the main source of the CD&SU population. In addition, referrals are being received from the Pediatric Departments of the different military hospitals in the area, including Fort Belvoir, Patuxent Naval Hospital, and Andrews Air Force Base. Each referral, including self-referral from parents, is made through the Department of Pediatrics of the U. S. Naval Hospital.

Each child is seen by a social worker, public health nurse, clinical psychologist, speech and hearing pathologist, education specialist, pediatrician, child neurologist, child psychiatrist and cytogeneticist. Consultation, if necessary, is obtained from various specialists from the U. S. Naval Hospital.

A minimum of two days is needed to evaluate a child. Consequently, the Clinic has been able to evaluate two new patients a week. Case conferences and parent conferences are held on each case. Referrals, if inidicated, are made to various community agencies since the Clinic is not designed, administratively and functionally, to provide service or therapeutic functions to the patients. As of April 12, 1966, 25 patients have been evaluated. By the end of the current fiscal year, we expect to see 21 more patients.

No individual research projects are being carried out as of April 13, 1966. However, we expect to initiate and pursue the attached descriptions of research projects before the end of the current fiscal year.

Serial No. HD-MR18

1. Children's Diagnostic and Study Unit
2. Clinical Research Branch
3. Building 125, NNMC

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Improving Attending Behavior in the Preschool Retardate

Previous Serial Number: None

Principal Investigator: Adam P. Matheny, Jr., Ph.D., Staff Fellow

Other Investigators: None

Co-operating Units: None

Man Years

Total:	.02 man years
Professional:	.02 man years
Others:	None

Project Description:

Objectives - To improve prelearning experiences in nursery school retardates by the application of operant conditioning techniques. Improvement in attending behavior can have a far-reaching beneficial effect in the acquisition of information.

Methods Employed - This project will employ operant conditioning techniques to improve prelearning experiences in nursery school retardates. The prelearning experiences - orienting behavior and selective attention - are those which represent precursors of the acquisition of more complicated perceptual and cognitive skills. These experiences are also those which are fundamentally poor in the young retarded child.

By improving these experiences, it is hypothesized that: 1) an improved span of attention can be shown by changes in subtest scores on psychometric scales; 2) improved orienting and attending behaviors will generalize to many other areas of behavior related to poor attention - e.g., hyperactivity; and 3) an improved learning rate can be shown in the retardate's acquisition of new learning skills involving visual materials.

The operant conditioning equipment is designed to present pictorial stimuli contiguously with a selected act by the child - such as pushing a lever. By manipulating the length of time between the child's act and the presentation of the pictorial stimuli, we manipulate the length of time the child waits (and attends) for something to happen visually.

Major Findings - This study is not yet in progress. As soon as the construction of the subject console and the control data system is completed, this project will be carried out.

Significance to Biomedical Research and the Program of the Institute - One of the primary manifestations of mental retardation is reduced learning capacity. If methods, such as operant conditioning, can be developed to increase one's ability to acquire information, then one of the missions of the Program would have been fulfilled.

Proposed Course of Project - These procedures can also be employed to monitor the effects of psychotropic drugs on the young retardate. Day-to-day variations of behavior can be recorded quite accurately by operant techniques.

Should this line of research prove fruitful, we will consider recording eye-movements as they relate to attending behavior. Consequently, we can expand the operant conditioning procedures to include modifying visual search strategies in the young retarded child.

Honors and Awards: None

Publications: None

Serial No. HD-MR19

1. Children's Diagnostic and Study Unit
2. Clinical Research Branch
3. Building 125, NNMC

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Behavioral Assessment of Neonates at Risk for Mental Handicaps

Previous Serial Number: None

Principal Investigator: Adam P. Matheny, Jr., Ph.D., Staff Fellow

Other Investigators: None

Co-operating Unit: Department of Pediatrics and Department of Obstetrics and Gynecology, U. S. Naval Hospital, National Naval Medical Center

Man Years

Total:	.2 man years
Professional:	.2 man years (1 person)
Others:	None

Project Description:

Objectives - To develop objective approaches to elicit responses that can be used as differential behavioral indicants of central nervous system integrity.

Methods Employed - This Program establishes "risk" indices for infants to be delivered at the National Naval Medical Center. Infants "at risk" are given controlled programs of auditory and visual stimulation, and specific behavioral responses are recorded. Comparisons of gross activity, changes in heartrate and skin resistance between "risk" and "normal" infants will establish early diagnostic indices for detecting mental subnormalities. Procedures and measures will accrue predictive validation by using a two-year longitudinal design. Diagnostic behavioral indices, when established, will be validated by correlational studies with presently obtained neurological information.

Major Findings - As soon as the multichannel polygraph and an instrumented infant crib are purchased, this investigation will be carried out in the Newborn Nursery of the U. S. Naval Hospital and the CD&SU.

Significance to Biomedical Research and the Program of the Institute - Application of effective therapeutic or remedial measures to prevent mental retardation hinges upon early detection of the problem. The ultimate goal of this study is to improve diagnostic procedures and diagnostic accuracy for detecting mental handicaps in the first year of life.

Should the procedures and measures gained from this study prove compatible with present clinical examinations, extensive screenings of large infant population can be made.

Proposed Course of Project - Daily examination of the experimental and control subjects will be carried out in the U. S. Naval Hospital Nursery. Longitudinal evaluations will be performed in the CD&SU up to the age of two years.

Honors and Awards: None

Publications: None

Serial No. HD-MR20

1. Children's Diagnostic and Study Unit
2. Clinical Research Branch
3. Building 125, NNMC

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Informational Discrepancies by Parents about Their Mentally Retarded Children

Previous Serial Number: None

Principal Investigator: Adam P. Matheny, Jr., Ph.D., Staff Fellow

Other Investigator: Joel Vernick, MSW, Clinical Social Worker

Co-operating Units: None

Man Years

Total:	0.1 man years
Professional :	1 professional 0.05, 1 professional 0.05
Others:	0

Project Description:

Objectives - The aim of this project is to develop means by which parents who are unrealistic about their mentally handicapped children can be: 1) circumscribed as a group, 2) studied to see what sociopsychological processes are in operation, and 3) given additional information which will help them to think and plan more realistically for their handicapped children's futures.

Methods Employed - This study is concerned with the amount of "distortion" or "discrepancy" hypothesized to exist between the factual information parents have (or have been given) about their mentally handicapped children and the future behavioral expectations they hold for these children. In the simplest form we are concerned with measuring the amount of discrepancy between what is diagnostically and prognostically true about the child and what the parents believe or expect to be true.

Prior to the parents' initial visit to the Clinic, the Social Worker will obtain information from the parents about 1) how much education they expect for their child, 2) what kind of occupation they expect for their child when grown, and 3) factual or diagnostic information the parents have gained about the child. The same types of questions are asked the parents during and six months after diagnostic and counseling procedures have been carried out.

Major Findings - Not enough data have been obtained to permit meaningful analysis. However, a pilot study, which was carried out at Johns Hopkins University by the Principal Investigator, suggested that: 1) lower socioeconomic groups had more realistic expectations for education and occupation goals for their retarded children than higher socioeconomic groups, 2) all groups became more factually and realistically oriented with increased ages of the children, and 3) all groups were more factually and realistically oriented with increased severity of mental retardation.

Significance to Biomedical Research and the Program of the Institute - By testing and retesting parents on their stated expectations for their children, we can see how they change or shift their expectations in relation to what is diagnostically and prognostically known about the children. We can, therefore, easily see where discrepancies occur and measure the attenuation of discrepancies.

Proposed Course of Project - This project will not only attempt to replicate the previous findings in the pilot study but will also determine whether the lower socioeconomic groups are more realistic about their handicapped children, or whether they expect less for their children with or without a handicap.

Honors and Awards: None

Publications: None

Serial No. HD-MR21

1. Children's Diagnostic and Study Unit
2. Clinical Research Branch
3. Building 125, NNMC

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Behavioral Assessment in a Free-Play Situation

Previous Serial Number: None

Principal Investigator: Adam P. Matheny, Jr., Ph.D., Staff Fellow

Other Investigators: Mrs. Laura McLaughlin, M.A., Education Specialist
Mrs. Alice Abramson, M.A., Education Specialist

Co-operating Units: None

Man Years:

Total:	.08 man years
Professional:	.08 (3 professionals at .0266/year)
Others:	None

Project Description:

Objectives - This project is designed to develop behavioral rating scales which can be scored by nursery school personnel for diagnostic purposes. The objectives of this research are: 1) to establish dimensions of free play behavior of nursery school children amenable to reliable and objective data analysis; 2) to link free-play behavior with other standardized psychometric techniques so that diagnostic material can be supplemented; and 3) to provide a set of observational tools for nursery school situations so that a child's learning baseline can be established reliably.

Methods Employed - To date, a behavioral rating scale has been devised which meets the criterion of having interobserver reliability. This scale is now being applied to population of mentally retarded children so that validation criteria can be met. The protocol consists of categories of behavior usually seen in a preschool population. Teachers observing the child in a free-play situation will rate the child on each of the categories. Ratings will be made along an eleven-point continuum. Certain categories of behavior will "anchor" the observations to more valid behavioral measures because these categories are analogous to standard psychometric items. All ratings will be made by at least two independent obervers - education specialists.

Major Findings - This study has been in progress for less than two months. Meaningful evaluation of data is not possible at this time.

Significance to Biomedical Research and the Program of the Institute - This research is directed toward extending the diagnostic tools and information applicable to the young mentally retarded child so that more accurate behavioral predictions can be made. With this approach, observational data can be used quantitatively, instead of qualitatively, for the purposes of differential diagnosis of mental handicaps and/or mental subnormality.

Proposed Course of Project - Ratings are made during the routine period in which the educational specialists observe a single child for diagnostic information. These observations are made in a fully equipped nursery - kindergarten room at the CD&SU. These independent ratings will be continued as a part of the observational period on each child who is evaluated in the Clinic.

Honors and Awards: None

Publications: None

Serial No. HD-MR22

1. Children's Diagnostic and Study Unit
2. Clinical Research Branch
3. Building 125, NNMC

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Mental Age Requirements for Conventional Audiometric Procedures, and Operant Conditioning Audiometric Techniques Employed with Preschool Retardates

Previous Serial Number: None

Principal Investigator: Susan S. Barr, M.S., Speech & Hearing Pathologist

Other Investigators: None

Co-operating Units: None

Man Years

Total:	.3 man years
Professional:	.3 (1 person)
Others:	None

Project Description:

Objectives - 1) to determine what mental ages are required of preschool retardates for a reliable and successful application of conventional audiometric testing procedures, and 2) to devise special audiometric techniques which will expand the age range of reliable and successful testing procedures.

Methods Employed - Each subject will be accompanied by the examiner's assistant (possibly the subject's mother) in a sound-proof room (IAC room built to the specifications of the American Speech and Hearing Association standards). Audiometric procedures and tests will include the following:

	Tests	Procedures	Data Expected
Part I.	Pure tone Audiometry (sine tones and warble tones)	Ear phones & Free Field	Audiogram (250-8000 cps)
Part II.	Speech Audiometry	Ear phones & Free Field	Speech awareness & speech thresholds reception

(a) Complex Speech (500-2000 cps)	Ear phones & Free Field	Awareness level
(b) White Noise (125-8000 cps)	Ear phones & Free Field	Awareness level

Instrumental conditioning based on a reward system will be employed for audiometric tests. This technique will teach the subject as much as possible by providing immediate reinforcement for each correct response to auditory stimuli. Procedures involved are listed in the following table:

	Sound Stimuli	Subject's Response
Part I. training	60-60 db, 250-8,000 cps (well above subject's threshold)	Assistant places subject's hand on dispenser handle each time stimulus is presented and initiates a reward.
Part II. training	60-60 db, 250-8,000 cps	Subject places hand on dispenser handle unassisted - this time the subject initiates his own reward
Part III. training to criterion	50-60 db, 250-8,000 cps	Subject meets criterion of having been conditioned
Part IV. testing	Threshold testing begins	1. Audiogram 2. Awareness level 3. S.R.T.

Major Findings - have not been established as yet, since this study has been in pregress for about a month.

Significance to Biomedical Research and the Program of the Institute - By gaining the information mentioned under "objecting," it is expected that: 1) Clinicians might know specifically what the retardates' mental ages should be before expecting reliable results from conventional audiometric procedures; 2) Audiologists might employ operant conditioning as a special technique in testing preschool retardates; and 3) Base-rate adjustments might be made on data showing a high incidence of hearing losses among retardates as indicated by other studies.

Proposed Course of Project - Should operant conditioning prove a more useful and effective means of determining auditory thresholds in preschool retardates, such procedures might be employed in a free-play situation with the nursery children at the Children's Diagnostic and Study Unit. In this situation, the subject would be conditioned for audiometric testing which he might well "administer" himself.

Honors and Awards: None

Publications: None

NICHD Annual Report
July 1, 1965 through June 30, 1966
Communications Program

Associate Director's Summary

During the year the Communications Program of the Institute made considerable progress.

The Public Information Branch continued the development of its work and was successful in placing material in major national magazines and the press. The Institute supplied the public information services at the annual meeting of the American Pediatric Society and the Society for Pediatric Research. Progress has been made towards developing a selective film guide in the field of reproduction.

The Public Information program of the Institute will be somewhat emphasized as a consequence of the interest in this function expressed by the Office of the Secretary.

Work in the Scientific Information Centers Program has gone forward with reasonable rapidity. In the latter part of the fiscal year, it was decided to make the Scientific Information Centers Branch operational and to appoint an Acting Director who would also be nominated for full incumbency. The preliminary work on the Scientific Information Centers in Aging and in Growth and Development is essentially complete and awaits only the installation of new computer facilities at NIH to become operational sometime during the present calendar year. Efforts are also being made to establish a third Scientific Information Center, in the area of Reproduction, and it now appears that staffing of this Center will be underway at some time during the present calendar year and certainly before the end of the next fiscal year.

The Interdisciplinary Conference Branch has had an active year. Several important conferences have been held in the areas of Reproduction, Growth and Development, and Mental Retardation. Two important all-Institute conferences have been held, and several areas of emergent interest are being given attention.

The Acting Associate Director for Communications indicated his intention to leave the Institute toward the end of the fiscal year, and a new Acting Associate Director, Dr. Georgia Perkins Reaser, was appointed effective May 2, 1966.

ANNUAL REPORT
Public Information Branch
Fiscal Year 1966

During fiscal year 1966, events which occurred at the Department level may well prove, in the long-run, to have exerted more influence on the information activities and services of the NICHD Public Information Office than did any events occurring within the Institute itself.

In a memorandum dated December 21, 1965, the Secretary of the Department of Health, Education, and Welfare indicated his hope that the Department's public information activities would "achieve a short-range goal of the best performance in Government and a long-range goal of performance unmatched anywhere."

The Secretary concluded his memorandum by saying: "...we have a duty to make information available and to tell the story of this Department. A sound information policy in the operating agencies cannot be built without the active interest and participation of the operating chief. He should include his public information officer in policy discussions. Only thus will the information program become an integral and significant part of the agency's activities. Therefore, the public information officer must be qualified to participate in the policy-making function of his agency and to run its public information service."

The announcement of this policy, by the Secretary, has been followed by a series of developments within the Department's Office of Public Information designed to upgrade the quality of the Department's public information activities. The developments include the relaxing of clearance procedures, the establishment of in-service training programs for public information personnel, and increased emphasis in television and radio coverage of Department news.

It is quite apparent that, whereas in the past the government information officer has frequently functioned primarily as "safety-man", he is now being asked to function more as "advance-man", and as a "middle-man"--the interpreter to many publics of the many new programs established by Congress and administered by the Department.

This is nowhere so apparent as in the fact that the number of Congressional, public, and press inquiries handled by the Information Office doubled in 1966 over 1965. As a result of these inquiries, NICHD-supported research and activities were featured in cover stories in Life, U. S. News and World Report, and Newsweek.

Other magazines with whom the Public Information Office dealt in the course of the year included: Good Housekeeping, Reader's Digest, American Baby, Parent's Magazine, Science, Harvest Years, Saturday Evening Post, National Observer, This Week Magazine, Electronics Magazine, Drug Research Reports, Time, Business Week, U. S. Medicine, Modern Medicine, Antibiotic News, Journal of the American Medical Association, Science Service, and Farm Journal.

Newspapers, wire and news services with whom the Information Office dealt included: Kansas City Star, United Press International, Christian Science Monitor, Medical World News, Medical Tribune, Navy Times, Newhouse Newspapers, Knight Newspapers, Washington Daily News, Washington Post, Trenton Times, Associated Press, New York Times, Philadelphia Bulletin, Philadelphia Inquirer, Baltimore Sun, and Atlantic City Press.

Press activity reached its height in April, when the NICHD Public Information Office planned and staffed a full-service working press room at the annual meetings of the American Pediatric Society and the Society for Pediatric Research in Atlantic City. This was the first time either society had had technical assistance in making press arrangements for their Annual Meetings, at which, of the papers presented, approximately half are supported with NICHD funds.

Other activities of major importance were:

The development, through a contract, of a selective film guide in early developmental biology, to be used in undergraduate and graduate research and training situations. Working with six consultants, the Institute's contractor developed a listing of approximately 500 films in early developmental biology, and viewed approximately 125 of the films. Twenty-five films have been selected for inclusion in the guide for their scientific and technical merit. The contract has been extended, and it is expected that the film guide will be published next fall.

Three issues of the news service to professional journals have been developed and distributed to approximately 60 professional and scientific periodicals interested in the disciplines relating to child health and human development. All of the journals receiving this news service requested that it be sent to them. The combined readership of these journals numbers several hundred thousand persons.

Seventy-five science writers who had indicated their interest in the fields of fertility, sterility, and population dynamics were sent the NACHD Council's recommendations concerning research in these fields. Subsequently, the NICHD press release concerning the letting of a contract to Princeton University to conduct a survey of family planning practices elicited considerable press interest from the AP, the UPI, the New York Times, the Washington Post, and the Washington Daily News.

To indicate the Institute's interest in the behavioral sciences, the tape of Miss Anna Freud's informal seminar on the work of the Hampstead Child-Therapy Clinic was edited, and, with Miss Freud's permission, was distributed widely to psychologists, psychiatrists, and other behavioral scientists and clinicians.

Fiscal year 1966 had its share of disappointments as well as successes:

The publications program, which was considered to be of primary importance, was not carried forward. Drafts were prepared both of a general NICHD pamphlet, to be illustrated with photographs of intramural laboratories and

activities, and of a pamphlet on the Reproduction Program. Considerable work needs to be done before these pamphlets--and others equally desirable--will be acceptable to the scientific and program staff.

As to the future, it is quite apparent that NICHD's Information Office is facing an extremely busy, and, hopefully, productive year in 1967.

Since many of the scientific areas with which the Institute is concerned are currently in the public spotlight--reproductive biology and infant mortality and health are examples--requests for service from news media, mass circulation magazines and scientific journals will continue to grow. The addition and rapid growth of the Institute's intramural laboratories--with the concomitant growth of scientific intramural staff--will also increase requests for service from the Information Office. This is already apparent in the number of manuscripts being cleared, the number of announcements and news stories made available to the NIH Record and local news media, etc.

Another more subtle factor makes it both possible and desirable to expand public information activities. Now midway through its fourth year, the Institute has begun to feel secure in its mission, and firm in its program commitments. Some of the uncertainties of the initial years have been weathered, and the Institute gives every indication of entering into an expansive and productive phase of its history. Hopefully, this stimulus will add to the productivity of the Information Office. Three new positions were added--an audio-visual specialist, an editorial clerk, and a writer-trainee. However, two key positions were vacated--one through resignation following maternity leave, and the second through the death of the incumbent. Both positions--that of senior science writer and of publications editor--should be filled as quickly as possible, and an additional secretary or clerk-typist engaged.

Contract Title: Film Guide on Early Developmental Biology

Contractor: Mental Health Materials Center, Inc.
New York, New York

Money Allocated: $22,400.00

The purpose of this contract is to prepare a selective guide on films in the field of reproductive and early developmental biology. As an integral part of the project, the Contractor, with the help of scientific consultants, identified all films relating to the field--approximately 500, and of these, the contractor and scientific consultants reviewed 125 for scientific excellence, technical superiority, and potential usefulness in teaching and research settings.

It is expected that approximately 25 films will be selected as outstanding educational and training films. The selective guide, for which the contractor is to prepare a manuscript, will include definitive reviews and discussion material.

NICHD Annual Report
July 1, 1965 through June 30, 1966
Interdisciplinary Conference Branch

During the fiscal year, NICHD has supported a total of twenty four conferences. Five of these were extramural awards. For these, the initiative and management were carried by the applicant institution. The balance of the conferences were sponsored or co-sponsored by the Institute, with the involvement of program staff in the planning, execution, and follow-up of each.

By program area, the intramural conferences included the following:

Reproduction Program

Recognition and Treatment of Neonatal Anoxia
Neonatal Fuel Metabolism
Developmental Pharmacology
Consultation on Population Research

Growth and Development Program

Conceptual and Methodological Approaches to Research in Outpatient Pediatric Departments
Learning Advisory Group Conference
Perceptual Development in Relation to Theories of Intelligence and Cognition
(co-sponsored with the Institute of Juvenile Research, Chicago, Illinois)

Mental Retardation Program

Mental Retardation Research Centers Program Consultation
Prevention of Mental Retardation through Control of Infectious Diseases
(co-sponsored with the University of Texas)
The Inter-American Workshop on Mental Retardation
(co-sponsored with the Department of Health, Education, and Welfare and the University of Puerto Rico)

No conferences were held by the Aging Program this fiscal year.

Two specialized conferences on Vocabulary Improvement and Development were held under the auspices of the Scientific Information Centers Branch; one for vocabulary in Growth and Development and Aging, and the second for Behavioral Sciences vocabulary development.

The Special Program Planning Committee, which was established in July 1963 under the chairmanship of Dr. Richard Smith to advise the Institute Director on long-range goals for the programs of the Institute, held four conferences during the year. The subjects of these were: Aging, Mammalian Pregnancy, Learning, and the Child in Society.

The function of the Interdisciplinary Conference Branch is to facilitate the multi-dimensional approach of this Institute for research in the totality of human development. The conference branch chief provides consultation to the Director and the scientific staff on the dynamics of group interaction so that the choice of conference structure can be closely interrelated with the objectives projected for each conference. The conference branch staff carries responsibility for establishing an appropriate and satisfying atmosphere for the achievement of the conference goals and arranges for the logistic support of the conference and the participants.

The experience of the Institute indicates that interdisciplinary conferences can be used to identify and clarify:

a. emergent areas where there is need to delineate a problem to make it operational for research,
   e.g., developmental pharmacology

b. technical problem areas where the field is already delineated, but a specific problem needs to be worked out,
   e.g., neonatal anoxia

c. continuing subject areas relating to problems which need to be studied again and again,
   e.g., learning

In the course of the three years of the conference program within this Institute, a card index has been developed with biographical information on the participants. This file, with more than 2000 names, represents a reservoir of talent covering a range of subjects in human development. It also serves as a resource file of younger investigators to be tapped for potential leadership roles in specific research projects.

The responsibilities of the Council Secretary and committee management functions have been absorbed by the Interdisciplinary Conference Branch during this year.

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Scientific Information Centers Branch

In May 1965, the Scientific Information Center for Aging was established in the Office of the Associate Director for Communications, NICHD and a librarian was employed as Head of the Center. In April 1966, the Scientific Information Centers Branch was established and an acting Chief and an acting Assistant Chief for the Branch were appointed. This Branch of the Communications Section consists of three specialized information centers:

1. Scientific Information Center for Aging
2. Scientific Information Center for Growth and Development
3. Scientific Information Center for Reproduction

These information centers were established to collect, store, retrieve and disseminate all information relative to the research in their respective fields. The objectives of these centers are fourfold; (1) to collect and store all information in their respective fields of interest, (2) to keep researchers current with the published literature in their fields by publishing monthly abstract journals and answering specific inquiries from the scientists in the field and publishing bibliographies on specific subjects, (3) to analyze and evaluate information so that evaluative reviews can be published periodically, and (4) to keep the researchers aware of the current work being done in their respective fields of study by means of states-of-the-arts lists.

The Scientific Information Centers Branch has compiled basic hierarchial vocabularies in the broad areas of research; Biology, Behavioral Sciences (Psychology and Sociology), and Related Sciences i.e., Biomathematics, Ecology, etc. within the fields of Aging, Growth and Development, and Reproduction. These vocabularies will be used to list the abstracts under two or more headings in the abstract journals where applicable. Two conferences have been held with extramural specialists in the fields of Aging, Growth and Development, and Reproduction for vocabulary improvement and finalization to assure the incorporation of the user's point of view.

An information storage and retrieval system has been designed for the programming of the N.I.H. 360 Computer which is to be installed by June 1966. The storage and retrieval system will select, sort and print out the abstracts in camera-ready copy for the abstract journals as well as provide bibliographic response to inquiries from the scientific community.

In December 1965, the Assistant Branch Chief came on duty and during March and April, 1966 three indexers and a secretary were employed and three more indexers will report within the next two months. The personnel of the centers are now learning the indexing methods and techniques to be applied and are searching the literature at the National Library of Medicine, the National Institutes of Health Library and the Department of Health, Education and Welfare Library for appropriate material to be listed in the

abstract journals.

The Scientific Information Centers for Aging, and Growth and Development will be operational in the fall of 1966 and abstract journals will be published the following month. The Scientific Information Center for Reproduction will become operational when sufficient personnel can be hired and trained.

Current awareness of states-of-the-arts lists will be compiled during the next fiscal year in the fields of Aging, Growth and Development, and Reproduction. These lists will contain summaries of research in progress in each of these fields of study.

By the end of 1967, the Scientific Information Centers Branch will have enough information stored and will have the staff available so the evaluative reviews can be developed in specific areas of the fields of Aging, and Growth and Development.

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Technical Reference Library

Since the last Annual Report, the activities of the NICHD Technical Reference Library have again been expanded to service the personnel which has more than doubled during the last Fiscal Year. (NICHD now has a staff of 350 compared to 154 which were serviced until June 1965).

The Library has again been partially reorganized: All periodicals were shelved alphabetically in 4A-10 and all books, paperbacks, directories, university catalogs and Legislative material were shelved in 4A-08. Where applicable, (subjects in which our Library had more extensive holdings) we added a cataloging system by color rather than by number, to identify books and paperbacks by subjects.

Cataloging has been continued - (238 books, orders and periodical-subscriptions) as have reference-activities at the NICHD Library itself, and liaison work with the main NIH and other Libraries. We fill approximately 107 requests for Xerox copies of articles or loans of periodicals and books from the NIH Library per month, but it is difficult to give an accurate account of these services. A few hundred grantee-reprints were received and cataloged. 32 bibliographies (202 pages) were prepared. 83 translations from 4 different languages were prepared (1250 pages) and several on-the-spot oral translations have been made.

The library staff of 3 has been reduced to a 2-man team again. In the nearest future, the NICHD Library will be moved to new premises in Bldg. 12A (Rooms 3027 and 3029) and before re-shelving, all books will be provided with book-pockets and book-cards for easier loan procedures.

The present NICHD Library holdings are about 250 periodicals and 2000 books and paperbacks.

One full-length scientific book (INITIATION OF LABOR, 241 pages, fully indexed, 108 illustrations) has been completed and distributed not only domestically but to many foreign countries. The first printing of this book was for 5,000 copies. It appeared as a soft cover number as well as a case-bound volume. In addition to the above circulation this book has gone to medical schools, medical libraries, etc., and many favorable responses have been received regarding its quality.

Another publication entitled SUDDEN DEATH IN INFANTS was prepared for final publication and will appear in print approximately the first part of July. This is the first full-length book of its kind to appear on this subject. It will be published both in casebound and soft cover. The number of copies, in addition to those to go on sale through the Government Printing Office, will be 11,000, plus 50 reprints of individual papers for each participant. This book is also fully indexed, carries 60 illustrations and is approximately 200 printed pages in length. This volume, because of the subject matter and the fact that it has been updated to include information current as recent as 1966, should have wide distribution and many requests have been received already from private libraries, individual pediatricians and others.

A third volume (NEONATAL RESPIRATORY ADAPTATION) has also been completed for final publication and should be ready for distribution shortly after the publication on SUDDEN DEATH. This also is a fully indexed, well illustrated (137) publication of 250 pages and will appear in both casebound and soft cover. The number ordered for our distribution is 5,000 copies. This is in addition to those to be placed on sale through GPO.

A fourth volume (PATTERNS OF HOUSING OF MIDDLE-AGED AND OLDER PEOPLE) was also prepared for final publication and is now being set in galleys. This is also indexed and illustrated. The number ordered on this is 5,000 copies, both casebound and soft covers.

NICHD Annual Report
July 1, 1965 through June 30, 1966
Office of Program Services

The reorganization of NICHD resulted in the establishment of four branches in the Office of Program Services. The former Office of Program Analysis, which had originally been assigned to Operations on a functional basis, is now the Program Statistics Branch of Program Services. Branch status has also been given to Facilities and Resources and to Extramural and Contract Management. Plans have been completed to activate the fourth branch, Biometry and Epidemiology, before the close of this fiscal year.

Continued emphasis has been given to strengthening the concept of Program Services as being the major supporting arm of the NICHD program staff. The growth of the NICHD programs and areas of responsibility are reflected in the branch reports which follow.

During the year there has been a continuous endeavor to establish and maintain close communication and working relationships both among the branches in Program Services and with the other organizational units of NICHD. In addition, special efforts have been made to maintain liaison with the Office of the Director, Extramural Programs, NIH, with the Division of Research Grants and the other Institutes and Divisions of NIH. One of our principal objectives is to maintain uniformity of policy and procedures within our four program areas; therefore, when definitive NIH guidelines are not available we are making an effort to develop our own.

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July 1, 1965 through June 30, 1966
Extramural and Contract Management Branch

Summary

The major objective of the Branch during the past year has been the continuation of effective administration and management of the Institute's various extramural activities. This has been a difficult task, considering factors such as: (1) reorganization of the Institute; (2) major changes in PHS and/or NIH policies and procedures effecting every area of the extramural program; and (3) the overall growth of the Institute's extramural program.

The reorganization of the Institute, including the consolidation of the scientific program areas from eight to four, and the establishment of program training officers and review committees, required a reassignment of the grants and contract management staff and adoption of new operational procedures for the Branch. This also necessitated the development of new working relationships, both within the Branch and between the Branch and the Scientific Program staff. The transition was handled with no adverse effect on the continuing administration of the extramural program.

Major policy changes affected both the Grants Management and Grants Administration Units. For example, the project period mechanism was implemented in the training grant program; the "interim cost-sharing" and, then, the new "cost-sharing" procedures were implemented in the research grant program; the format of the approval list and notice of award was revised for both training and research grants; and the encumbrance procedure was completely changed in the fellowship program. All of these required procedural changes in the operations of the Branch.

The growth of the extramural program has also had an impact on Branch operations. The Branch will process awards and contracts amounting to approximately $45.9 million, an increase of approximately $10.4 million over FY 1965.

Although we still have problems which must be solved, we feel we have continued to make our operation function at a high level of effectiveness.

Programs

Research Grants

During FY 1966 we expect to award 1139 regular research grants for $34.5 million, an increase of 160 grants and $8.0 million over FY 1965. The total consists of approximately 640 non-competing continuations for $19.3 million, 144 renewals for $4.8 million, 284 new grants for $9.0 million and 71 supplementals for $1.4 million. We awarded one clinical research center grant for $0.2 million and transferred $3.8 million to the Division of Research Facilities and Resources for the General Research Support Grant Program.

Training Grants

During FY 1966 we expect to award 124 graduate training grants for $7.8 million, an increase of 36 grants and $2.4 million over FY 1965. The total consists of approximately 71 non-competing continuations for $4.2 million, 15 renewals for $1.0 million, 35 new grants for $2.5 million and 3 supplementals for $0.1 million. In addition we expect to obligate $0.1 million for scientific evaluation grants.

Fellowships

During FY 1966 we expect to award 156 regular fellowships for $1.3 million, an increase of 34 and $0.3 million over FY 1965. The total consists of approximately 107 post-doctoral fellowships for $0.8 million and 49 special fellowships for $0.5 million; or by type of awards, 33 non-competing continuations for $0.3 million, 20 renewals for $0.2 million and 103 new for $0.8 million.

Research Career and Research Career Development Awards:

During FY 1966 we expect to award 80 research career and research career development awards for $1.6 million. This represents an increase of 3 awards and $0.1 million over FY 1965. The total consists of approximately 71 research career development awards for $1.4 million and 9 research career awards for $0.2 million. By type of award, the total consists of 61 non-competing continuations for $1.3 million, 6 renewals for $0.1 million and 13 new awards for $0.2 million.

Contracts

During FY 1966 we expect to process 30 research contracts and reimbursable agreements for $1.1 million. This includes new and renewal contracts and represents a decrease of 1 contract and $0.3 million from FY 1965.

Staffing

We expect to have 35 positions filled in the Branch during FY 1966, an increase of 10 positions over FY 1965. The additional positions were to employ one financial management specialist, one grants and contracts management specialist, three grants and contracts management assistants and five clerical personnel. However, three of these positions will not be filled until near the end of the fiscal year. The Branch has operated throughout the year with between three and five other positions unfilled because of attrition and the difficulty encountered in recruiting replacements.

Accomplishments and Objectives

During FY 1966 the Branch has re-aligned the grants management staff to maintain a direct relationship with the Institute's reorganized scientific program areas; revised and/or developed procedures to implement a seemingly endless stream of major policy changes involving the extramural programs; effected a more comprehensive and detailed review of applications for new

and continuation grants and awards; and generally has sought, and will continue to seek, a higher degree of competence in the overall administration and management of the extramural program.

In seeking improvement, the Branch has increased the number of operating positions and added two staff positions to the Branch Chief's office. The increase in operating personnel will bring the individual workload to a more acceptable level and, therefore, raise the quantity and quality of the pre-award review and processing, as well as the managing of on-going projects. We also hope that this will enable the grants and contract management staff to take more field trips and work even closer with our scientific staff to provide guidance for our grantees. Every effort will be made to identify and resolve management problems before they become extreme.

A financial management staff position has been added to the Branch Chief's office and action has been initiated to improve the forecasting of fiscal requirements and budgeting projections. We are reevaluating our own fiscal records, while assessing the possibilities of utilizing to a greater extent the available statistical records from the Division of Research Grants, in an attempt to develop the most reliable fiscal record system for our needs. A more accurate system is essential to meet the requirements of a much tighter fiscal situation in the future.

A grants management staff position has been established to serve as a coordinating point in the identification, interpretation, and implementation of new and revised policies issued as guidance to the various extramural programs. We have taken action, and will continue to formalize, wherever possible, our operating procedures to assure consistency in the interpretation and implementation of new or existing policy.

In a further effort to improve our efficiency, we have initiated a comprehensive study and evaluation of the Branch operations by a team from the Management Policy Office, Office of the Director, NIH. The study team will analyze our current procedures and make recommendations for improvements.

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Facilities and Resources

The Facilities and Resources Branch of Program Services Division has directed its efforts during the past year to the following activities: (1) coordination of plans for construction and renovation of space for intramural research, with particular attention to the development of a Program of Requirements for the projected Institute laboratory building to be constructed on the NIH campus; (2) liaison with the Division of Research Facilities and Resources (DRFR) in areas of mutual interest, especially the DRFR General Clinical Research Centers Branch; (3) consultation services relating to facilities and resources to Institute program directors, staff members and institutions seeking research support; (4) collection of information about research facilities and resources across the country which are of special pertinence to the NICHD mission.

The Mental Retardation Diagnostic and Study Unit, located at the National Naval Medical Center, has been completed and is presently in operation. As of the writing of this report, design was 30% complete for the Biomedical Laboratories which will be utilized in conjunction with the Study Unit. It is hoped that construction can begin shortly after July 1, 1966. Occupancy can then be anticipated by April 1967, possibly earlier.

Planning for the construction of a joint NINDB-NICHD Reproductive Biology Research facility at El Centro Medico (the new campus of the University of Puerto Rico's School of Medicine), has proceeded. The Architect/Engineer has been hired and it is expected that tentative drawings will be finished by November, with actual construction scheduled to begin in the summer of 1967.

In February 1966, approval was granted for the use of $200,000 from the funds authorized for the joint facility described above, for the rehabilitation of 600 acres released by the Navy, approximately 10 miles from El Centro Medico, at Sabana Seca. This area is to be renovated and enclosed by fencing, and is to be used as a monkey breeding facility and for behavioral science research. It will be shared by NINDB and NICHD and will function as an adjunct to the facility at El Centro Medico.

The Gerontology Building, recently under construction in Baltimore, will be operated by the Aging Program. Research will be conducted in collaboration with the Maryland Commission and the Baltimore City Hospitals. The building is scheduled for completion in the late fall of 1967.

The work of planning for the new NICHD laboratory facility is continuing. The final version of the Program of Requirements, arrived at through the close collaboration of DRS (Division of Research Services) and FRB, NICHD, has been submitted for approval. Visits to a number of laboratory facilities on the NIH reservation, and to commercial laboratories and non-profit institutions were made by FRB staff in connection with the planning of certain portions of the building such as virology, ultrasound, tissue culture.

In addition, consultants who had previously advised on the functional program statement were asked to review and formally comment on the space directive and design criteria. Review of the P.O.R. by and advice from a number of NIH experts was also obtained. It is hoped that approval of this document will permit the hiring of the Architect/Engineer by July 1966.

Liaison with the Division of Research Facilities and Resources has continued. FRB has participated in three site visits on HRF and Clinical Research Center applications of interest to NICHD and has represented NICHD at the General Clinical Research Centers Review Committee, Animal Resources Advisory Committee, and Scientific Review Committee meetings, as well as at the NARRC and NAHRFC. Formal provision of information regarding NICHD program pertinence and interest in HRF applications is coordinated through this office.

Consultative services to staff members and to prospective grantees have been a continuing function.

A request from the NACHHD planning committee for information about behavioral science research space in the U.S. has been received and preliminary work has begun toward obtaining the information desired.

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Program Statistics Branch

NICHD Extramural Grant Program

The Program Statistics Branch has completed the coding and storing on IBM punch cards of basic administrative and fiscal information for all NICHD research and training grants, career program awards, and fellowships and is now fully current. The frequency of regular reporting, which has been on an inter-Council basis, is being stepped up to a monthly basis beginning with the March report. Moreover, plans are under way to expand our data bank to include contracts as well as additional detail on grants (such as departments, trainee data, etc.)

Analyses of these data have been prepared for the use of program staff, as well as many special non-recurring reports to fill specific needs. In addition, we have provided service to Institute staff as a clearing house of information on grants and applications of other institutes.

Our most recent data show that the NICHD grant portfolio continues to grow. In March of 1965 active grants of all types numbered 1126; by March 1966 they had increased to 1352, a gain of one-fifth. The dollar amount of grants, meanwhile, rose from 31 million to 41 million, an increase of nearly one-third.

Fellowships and training grants showed the largest increase - each of these programs was more than half again as large in March 1966 as a year earlier. The research grant program grew by more than a fourth during this period. The training programs - training grants, fellowships, and research career program - now make up almost one-fourth of the Institute's grant program.

About eighty percent of the NICHD extramural program is concentrated in two program areas - Reproduction and Growth and Development; the Reproduction Program accounts for approximately half the total Institute grant program, and the Growth and Development Program about a third.

Our Institute supports projects in all states but three (Alaska, Idaho, and Wyoming) and in 18 foreign countries. New York State leads the list as the largest recipient followed in turn by California, Massachusetts, Pennsylvania, and Maryland.

Five institutions - the University of California, Johns Hopkins, Harvard, Yeshiva, and Western Reserve - are currently receiving more than $1 million each in NICHD support. Among them, they account for almost one-fifth of the Institute's grant funds.

The staff of the scientific group within the Program Statistics Branch has increased during the year by the addition of a psychologist-program analyst and a clerk-typist to the previous staff of two biologists, and recruitment for two additional biologists is currently underway. This expansion has been necessitated by the steady increase in work load in all areas.

Work on the scientific content classification of Institute-supported research is well advanced and we hope to have all FY 1966 projects coded shortly after the end of the fiscal year when the last FY 66 awards will have been received. This involves classification by discipline, by nearly 200 research topics in biomedical, psychological and socio-environmental fields, by diseases, by anatomical sites, substances, and types of organism. The system is designed for punched-card storage, print-out, and retrieval, and having the coded information available on cards and in print-out form will facilitate the preparation of material for reply to the wide variety of requests received, as well as provide data for program analysis.

We have had a steadily increasing number of requests for information about NICHD, NIH, or other agency support in numerous areas of Institute interest. Some examples of topics on which information has been provided include birth defects, cytogenetics, nutrition, learning, teratogenic action of drugs, speech and hearing, and the effects of religious education on moral development. In addition, we have provided information in answer to recurring inquiries about current research and training support relevant to mental retardation and about activities in the population field. In the latter area, a survey of Current Research on Fertility, Sterility and Population Dynamics active as of March 1965 was completed, including projects supported by all agencies reporting to the Science Information Exchange. Also, data have been provided on NIH and NICHD support of population activities for fiscal years 1964, 1965, and 1966. As additional staff members are acquired and trained, we plan to undertake the important but massive task of reviewing all NIH support in this field on a historical basis in order to provide trend data.

The staff has continued to have responsibility for the assignment of all new applications for research, training, fellowships and career program awards to the Institute's Program Areas, and over 300 applications were reviewed and assigned in the month of March alone.

The recent adoption of Program Planning Budgeting by NIH has involved our group in the development of program data applicable to target areas appropriate to the Institute's interests and activities. It is anticipated that work in this area will expand as the Program Budgeting concepts are further developed.

Growth of NICHD Grant Program, 1965-1966

Type of grant	Number of Grants			Amount of Funds		
	Active March 1965	Active March 1966	Percent increase	Active March 1965	Active March 1966	Percent increase
Total	1,126[a/]	1,352[a/]	20.1	$31,153,370[a/]	$40,902,876[a/]	31.3
Research	883	1,035	17.2	24,867,312	31,624,448	27.2
Training	80[a/]	102[a/]	27.5	4,175,429[a/]	6,497,447[a/]	55.6
Fellowships	91	137	50.5	730,465	1,176,923	61.1
Res. Career Prog.	72	78	8.3	1,380,164	1,604,058	16.2

a/ Includes two Chairman's grants not assigned to a program area.

NICHD
Program Statistics Branch
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NICHD Grants, Active March 1966

By Program Area and by Type of Grant

Program Area	Number of Projects					Current Support				
	Total	Res.	Tr.	Fell.	RCP	Total	Res.	Tr.	Fell.	RCP
Total	1,352[a/]	1,035	102[a/]	137	78	$40,902,876[a/]	$31,624,448	$6,497,447[a/]	$1,176,923	$1,604,058
Reproduction	720	568	42	75	35	18,705,778	14,337,097	3,017,761	625,095	725,825
Growth & Develop.	448	331	39	46	32	13,876,214	10,465,550	2,346,973	415,411	648,280
Aging	84	63	9	7	5	4,121,908	3,379,420	577,715	64,286	100,487
Mental Retardation	98	73	10	9	6	4,038,976	3,442,381	394,998	72,131	129,466

Percent Distribution by Program Area

Program Area	Number of Projects					Current Support				
	Total	Res.	Tr.	Fell.	RCP	Total	Res.	Tr.	Fell.	RCP
Total	100.0[a/]	100.0	100.0[a/]	100.0	100.0	100.0[a/]	100.0	100.0[a/]	100.0	100.0
Reproduction	53.3	54.9	41.2	54.7	44.9	45.7	45.3	46.4	53.1	45.2
Growth & Develop.	33.1	32.0	38.2	33.6	41.0	33.9	33.1	36.1	35.3	40.4
Aging	6.2	6.1	8.8	5.1	6.4	10.1	10.7	8.9	5.5	6.3
Mental Retardation	7.2	7.1	9.8	6.6	7.7	9.9	10.9	6.1	6.1	8.1

Percent Distribution by Type of Grant

Type of Grant	Number of Projects					Current Support				
	Total	RP	GD	AG	MR	Total	RP	GD	AG	MR
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Research	76.6	78.9	73.9	75.0	74.5	77.3	76.6	75.4	82.0	85.2
Training	7.5	5.8	8.7	10.7	10.2	15.9	16.1	16.9	14.0	9.8
Fellowships	10.1	10.4	10.3	8.3	9.2	2.9	3.3	3.0	1.6	1.8
Research Career Prog	5.8	4.9	7.1	6.0	6.1	3.9	3.9	4.7	2.4	3.2

a/ Includes two Chairman's grants not assigned to a program area.

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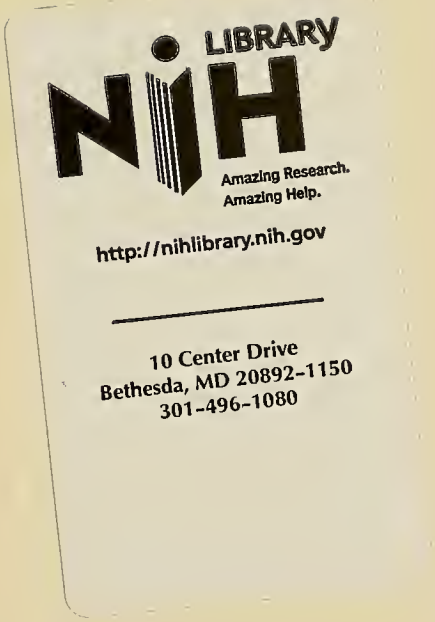
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